

Rain-Induced Fruit Cracking in Sweet Cherry (*Prunus avium* L.).

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Declaration

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Penelope Measham

Abstract

Production of sweet cherries (*Prunus avium* L.) around the world is centred on achieving high quality, blemish free fruit. One of the main concerns to Australian cherry growers and limitations to production is the unpredictable nature of rain-induced fruit cracking, which occurs in the later stages of fruit development and renders fruit unsaleable in markets that attract premium fruit prices. Control of cracking remains unreliable and the underlying mechanism is not yet fully understood. This thesis examines cracking from a tree water relations perspective, starting with patterns of crack development, the relationship of rainfall and fruit properties with cracking incidence, and concludes with an exploration of the underlying mechanisms resulting in crack development.

Cracking takes three distinct forms; stem end cuticular fractures, apical end cuticular fractures and large cracks, usually deep into the pulp, on the cheek of the fruit. This study has demonstrated that although all three types developed in the three-week period prior to commercial harvest, varieties displayed different levels of total cracking and distinctly different proportions of each crack type. Overall the extent of cracking was strongly controlled by season. While initial development of cracks coincided with rainfall, no relationship between amount of rain and incidence of cracking was found. There were also relationships between both crop load and tangential stress of the fruit skin with crack type and incidence.

Influx of water to the fruit via the vascular system was recorded after rainfall, prompting an investigation of the influence of water uptake both via the vascular system and directly across the skin. Application of excess water to simulate

rainfall, only to the root-zone induced large cracks in the side of the fruit. Application of water at a similar rate to the canopy induced cuticular cracks localised around the stem and apical end of the fruit with no increase in deep side cracks. This finding suggested different water uptake mechanisms driving development of side cracks and the shallower cuticular cracks at the ends of the fruit. An exploration of the driving forces responsible for vascular entry of excess water into the fruit proposed influx via the pedicel phloem and supported a role for adjacent leaves in control of water movement under conditions likely to initiate deep side cracks.

The findings establish mode of water uptake into the fruit as the main determinant of crack type in a susceptible variety. Future management of cracking needs to consider both varietal and seasonal factors, and may need to be variety or crack type specific.

Dedication

This thesis is dedicated to all wives, mothers and children who are led to believe they are worthless.

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Preface

This thesis is composed of papers, which have been either published or submitted, or are in preparation for publication, to refereed journals. The structure of the thesis is such that these research papers have been incorporated as research chapters, with the following changes:

- References have been removed and aggregated into a single list appearing at the end of the thesis;
- In-text citations of chapters are referred to as chapters not papers.
- Abstracts have been removed and incorporated into the thesis abstract;
- Acknowledgements have been removed and incorporated into the thesis acknowledgements;
- Figures and tables have been renumbered in line with thesis chapter numbers;

The research chapters document research undertaken between November 2005 and November 2008. Two chapters refer to small trials undertaken in early 2005. An introductory chapter provides the PhD project background and justification, followed by a literature review chapter outlining current approaches to the cracking problem. A chapter outlining general methodology used for many of the trials is included, and the discussion chapter integrates the discussion sections presented in each of the research chapters. This project has significant industry focus, and as such, a final chapter outlining recommendations to industry is also included.

Publications Arising From This Project

Refereed Journal Articles

P.F. Measham, S.A. Bound, A.J. Gracie, and S.J. Wilson (2009) “Incidence and type of cracking in sweet cherry (*Prunus avium*. L.) are affected by genotype and season.” Crop and Pasture Science 60 (10); 1002-1008

P.F. Measham, A.J. Gracie, S.J. Wilson and S.A. Bound (2010) “Vascular flow of water induces side cracking in sweet cherry (*Prunus avium*. L.).” Advances in Horticultural Science 24 (4); 243-248

P.F. Measham, A.J. Gracie, S.J. Wilson, and S.A. Bound “Diurnal drivers of vascular flow in relation to rain induced cracking of sweet cherry (*Prunus avium*. L.).” Prepared for submission to Advances in Horticultural Science

P.F. Measham, S.A. Bound, A.J. Gracie, and S.J. Wilson, “Low crop load promotes fruit cracking in sweet cherry (*Prunus avium*. L.).” Prepared for submission to Scientia Horticulturae

Non-Refereed Articles, Scientific Conference Papers and Industry Presentations

P.F. Measham (2010) „Directions in cherry cracking research’, Fruit Growers Tasmania Communication, Fruit Growers Tasmania, Hobart, Tasmania

Measham, PF, „Cherry Cracking in Tasmania’ (2010) Poster presentation to Fruit Growers Tasmania „Harvesting Success’ Annual Conference, 20-23 May 2010, Launceston, Tasmania

P.F. Measham, A.J. Gracie, S.A. Bound, and S.J. Wilson „An alternative view of rain induced cracking of Sweet Cherry (*Prunus avium*. L)’ Proceedings of the ISHS Sixth International Cherry Symposium (2009), Acta Horticulturae (In Press)

P.F. Measham, A.J. Gracie, S.A. Bound, and S.J. Wilson (2009) „An alternative view of rain induced cracking of Sweet Cherry (*Prunus avium*. L)’ Oral presentation to the ISHS Sixth International Cherry Symposium, 15-19 November, Renaca, Chile

P.F. Measham, S.A. Bound A.J. Gracie, and S.J Wilson, (2009) „Rain induced Sweet Cherry fruit cracking’, Proceedings of the HortExpo, 40th National Cherry Conference, 3-6 August 2009, Hobart, Tasmania

P.F. Measham, A.J. Gracie, S.J. Wilson, and S.A. Bound (2008) „An alternative view of rain induced Sweet Cherry (*Prunus avium*. L) fruit splitting ’, Proceedings

of the National and Trans Tasman Horticultural Science Conference, 21-23 July, Gold Coast, Queensland

P.F. Measham, A.J. Gracie, S.J. Wilson, and S.A. Bound (2008) „Rain induced Sweet Cherry (*Prunus avium*. L) fruit splitting: An alternative view’, Proceedings of the 39th National Cherry Conference, 7-10 August, Griffith, New South Wales,

P.F. Measham (2008) „Varietal influence on fruit cracking’, Nutrition and Fruit quality workshop, Huon Fruit Growers Group, as part of the Serve-Ag/Landcare sustainable Agriculture Project

P.F. Measham (2008) „Fruit Cracking & Water Retention’, Oral Presentation to Fruit Growers Tasmania May Conference, Cradle Mountain National Park

P.F. Measham (2006) „Fruit Cracking & Quality Issues’, Oral Presentation to Fruit Growers Tasmania May Conference, Freycinet National Park

A.J. Gracie, P.F. Measham, S.J. Wilson, P.H. Brown, (2006) „Causes of rain induced cracking of sweet cherries’, Proceedings of the 27th International Horticultural Congress, 12-20 September 2006, Seoul, Korea

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Abbreviations

π	Osmotic potential
T	Turgor pressure
CT ₅₀	Turgor pressure at which 50 % of fruit cracked
Ψ	Water potential
Ψ_F	Fruit water potential
Ψ_L	Leaf water potential
Ψ_S	Spur xylem water potential
$\Psi_L - \Psi_S$	Potential gradient between the leaf and xylem
$\Psi_F - \Psi_S$	Potential gradient between the fruit and branch xylem
TS	Tangential stress of the fruit skin
BCSA	Branch cross-sectional area
TCSA	Trunk cross-sectional area
AEDT	Australian Eastern Daylight Time
LR	Removal of spur leaves to expose fruit
LP	Spur leaves tied back to expose fruit
VPD	Vapour pressure deficit
e_{sat}	Saturation vapour pressure
e_{air}	Air vapour pressure
LVDT	Linear variable differential transducer
ϵ	Strain

Chapter 1

Thesis Scope and Justification

Introduction

Sweet cherry production is an economically important sector in many regions of the world. The unpredictable nature of rain induced fruit cracking is not yet fully understood and has significant implications for production and industry growth. This chapter provides an overview of cherry production at a global, national and state level. Also included is a brief description of cherry fruit growth and development and an introduction to rain induced sweet cherry fruit cracking and the current strategies employed to manage this problem.

Sweet Cherry Production

Sweet cherries are produced commercially by more than sixty countries worldwide with an estimated production of nearly two million tonnes of fruit (FAO 2008). The majority of sweet cherries produced are destined for the fresh fruit market, and are supplied from Northern Hemisphere regions. Turkey is currently the largest cherry producer with 30 000 hectares under harvest, producing 392 000 tonnes of fruit (10.3 tonnes/hectare) (Table 1).

Although production is considerably higher in the northern hemisphere, the European markets are currently undersupplied. Therefore opportunities exist for counter seasonal exports by southern hemisphere regions, including Australia. Chile is the largest producer of fruit in the southern hemisphere, having increased production by 34% in the last ten years to 35 000 tonnes. Australia follows with 12 500 tonnes, with a 46% increase in production and the highest increase in productivity from 4.2 to 6.9 tonnes/hectare over the last ten years (Figure 1). Only a small amount of the total fruit production is used in processing, with canning being the most viable option.

Table 1 Comparison of area under production, yield and productivity of sweet cherries (2007) between the largest producing Northern and Southern Hemisphere countries (FAO 2008).

Northern Hemisphere Production			
Country	Area (ha)	Yield (t)	Productivity (t/ha)
Iran	34 000	225 000	6.6
Italy	29 713	145 126	4.9
Spain	23 000	67 600	2.9
Turkey	30 000	392 001	13.0
USA	33 000	270 000	8.2
TOTAL	149 713	1 099 727	7.1
(Average)			
Southern Hemisphere Production			
Country	Area (ha)	Yield (t)	Productivity (t/ha)
Argentina	1 400	6 800	4.9
Australia	1 800	12 500	7.0
Chile	7 500	35 000	4.7
New Zealand	580	1 900	3.3
Peru	85	515	6.0
TOTAL	11 365	56 715	5.2
(Average)			

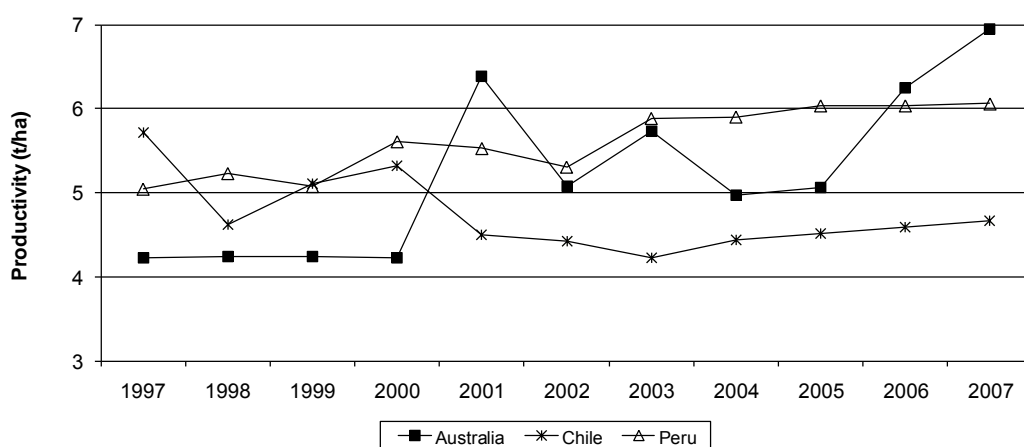


Figure 1 Productivity trends in the largest producing southern hemisphere regions over the 10 years to 2007.

The Australian Cherry Industry

Australian sweet cherry production contributes less than 1% of total world production. However, Australia is the second largest producer of sweet cherries in the southern hemisphere. In 2007 approximately 12 500 tonnes of fruit was harvested from 1 800 ha of orchards (FAO 2008). Production occurs commercially in the states of Tasmania, Victoria, New South Wales and South Australia. Australia has experienced the largest increase in productivity of all southern hemisphere growing regions. The reputation of Australian produced cherries as premium grade that satisfy stringent international market specifications has enabled the industry to acquire and supply overseas markets. Australia is well placed to expand existing export contracts and access new international markets. It was estimated that at least 10% of all cherries produced in Australia in 2004 were exported (Frias 2005) with the potential for this level to increase in the future.

Current export markets include regions in Asia, Europe, and North America. The Taiwan market has recently become inaccessible due to new fruit fly regulations; however Tasmania and the Riverland producing regions are exempt as they qualify for a fruit fly free status. The biggest competitor to Australia for counter season export markets is Chile where production levels are high and labour is relatively cheap but Australia has been able to maintain its competitiveness by focussing on fruit quality.

National and state cherry industry bodies are actively seeking to reduce any barriers to export. These barriers include the relatively high cost of production,

availability of air freight over the harvest period (which falls over the Christmas holiday season) lack of marketing, understanding consumer demands and biosecurity issues (Ranford 2008). A strategic plan to address these issues has been developed, and a National Industry Development Officer position created (HAL 2008). Practical research focussing on these key areas is being supported through industry levies and Horticulture Australia Limited (HAL 2008).

There is a continued push to increase domestic consumption of cherries in Australia through the establishment of a national marketing strategy in 2004, focussed on the health benefits of consuming fresh fruit and vegetables (HAL 2008). An increase in domestic consumption, combined with ongoing efforts to seek new market opportunities, such as counter seasonal marketing in the United States of America and Japan, while maintaining existing exports, has culminated in a 50% increase in cherry production since 1997 (FAO 2008).

The Tasmanian Cherry Industry

The Tasmanian Cherry Industry is rapidly growing, and is the largest exporter of cherries from Australia to Taiwan, Thailand, Singapore and the United States of America (TPCP 2008). Potential exists for increasing both the domestic and export markets: furthermore recent access has been granted into the lucrative Japanese market. Tasmania's fruit fly free status and extended season provide the state with a competitive edge over mainland counterparts in securing those markets. Indeed export levels have risen from 10 to 25% of total production. This optimistic outlook has seen an increase in plantings in Tasmania over the last 10 years. The cherry industry is a significant contributor to the Tasmanian agricultural sector with the farm gate value estimated to rise to \$25 million by 2010, an increase of 80% since 2000 (DPIW 2008).

These growing enterprises in Tasmania are situated in areas previously dominated by apple production, areas that provide an optimum climate for cherry production. These regions include the South-East district, Coal River Valley, Derwent Valley, Huon Valley, Tamar Valley, and some areas along the East Coast and North-West Coast. Most of the growth over the last decade has occurred in the Derwent Valley with large, new cherry orchards established in response to a Department of Primary Industries, Water and Environment (DPIWE) report (1991) on prime sites for stone fruits (Boucher 1991).

All the first class fruit produced in Tasmania has been suitable for the fresh fruit market and given the late production season, market opportunities on the mainland are more available than for mainland produced fruit. The Primary

Industry Department of the Tasmanian Government has identified the South Island of New Zealand as the only major domestic and overseas market competitor for late season cherries of comparable quality (DPI 1988, Boucher 1991).

Reliable production of blemish free, high quality fruit that meets market requirements is paramount to the continued success of the industry. The inability to predict the percentage of fruit classed as waste has impeded the development of a reliable market. Cracked fruit fall into this category, and given that the incidence of cracked fruit can be as high as 50% for any given year (pers comm. H. Hansen 2004), this has created a sizeable contract risk.

Cherry production is a capital intensive industry and considerable investment has been made in Tasmania to achieve the current level of production. However, the high incidence of rain-induced cracking has provided the major challenge faced by the local industry to date. Tasmania, with its long growing season is naturally prone to weather patterns that bring summer rainfall events and an increased risk of cracking. This cracking is unpredictable and results in significant economic and market losses.

Sweet Cherry Description

The sweet cherry (*Prunus avium*. L.) belongs to the Rosaceae family, sub-family Prunidaecea (Rodrigues *et al.* 2008). It is believed to have originated from the regions between the Black and Caspian Seas of Asia Minor. Seed dispersal by birds carried it to Europe, where the earliest cultivation of sweet cherry is reported (Webster and Looney 1996). Further spread to North America via English colonists occurred in the seventeenth century. To date, at least a hundred varieties of sweet cherry are recorded, and these are being commercially produced in more than sixty countries (Webster and Looney 1996).

Cherry trees are deciduous and are naturally vigorous, with strong apical dominance. Commercial production has been enhanced by the use of dwarfing rootstocks in order to limit tree height and encourage fruit set (Bargioni 1996). Advances in the development of dwarfing and semi-dwarfing rootstocks over the last decade, has also led to increased quality from plantings of high density cherry orchards (Robinson 2005).

Flowering occurs in racemose clusters of two to five flowers on short spurs. The distal bud is vegetative, providing continued spur growth into the next season. To achieve a viable fruit crop, a high proportion of flowers compared with other deciduous fruit must remain for fruit development (Thompson 1996), and thinning of fruit is not always necessary. Cherry fruit are also comparatively quick to mature and, depending on growing climate, only take two to three months following anthesis to reach harvest maturity (Thompson 1996). While some varieties are self compatible most are self incompatible and sufficient fruit set is

therefore dependant on cross pollination by bees. Twelve incompatibility groups have been identified (Webster and Looney 1996) and these often determine the layout of an orchard. Polliniser trees must be interspersed throughout the orchard to achieve desired fruit set for most commercial varieties.

The sweet cherry fruit is a glabrous, round or heart shaped fruit with a long pedicel, borne on short spurs from older wood (Thompson 1996). It is classified as a drupe, a simple fruit derived from the pericarp only, with a stony endocarp, and the ovary wall fleshy at maturity (Raven *et al.* 1992).

Rain Induced Cherry Cracking

Commercial production of cherries around the world is centred on achieving high quality fruit. As yet no region is free from the risk of rain induced fruit cracking (Christensen 1996).

Cracking occurs in the weeks leading up to harvest when fruit are mature (Sekse 1995b, Lane *et al.* 2000), and is usually associated with a rainfall event. This has proven unpredictable in terms of exact timing and extent of damage. Anecdotal evidence suggests that physical losses can be as great as 80% of harvestable yield, with recorded losses reaching 63% (Cline *et al.* 1995a). Yield losses beyond 20 - 30% are considered uneconomical to harvest (Hanson and Proebsting 1996).

The issue of cherry cracking is a major concern for all cherry producing regions, not only in terms of economic viability, but also for reliability of contracted export supply. Much of the research into resistant or susceptible varieties has been undertaken in the northern hemisphere, with southern varietal selection for cracking properties based on grower opinions, and performance in other regions. An added risk is that many small cracks or fractures go unnoticed when visually inspected at harvest, making produce susceptible to post harvest rots during transport.

Cherry cracking is often associated with rainfall events. Thus the prominent theory has been that direct water entry via the fruit skin surface, affecting cuticle and flesh properties, was the reason behind cracking (Christensen 1996). An alternative theory has involved the whole plant, where oversupply of water to the

fruit is thought to arise via normal internal water uptake mechanisms in the tree (Sekse 1995b). An internal excess of water is believed to increase the tensile forces acting on the fruit skin causing it to burst. However, the method of water entry into the fruit responsible for the build up of tension, and resultant cracking is not clear.

Current management techniques used to limit cherry cracking have all attempted to reduce water uptake through the fruit surface. This is based on the premise of the prominent theory, as outlined above, that water uptake resulting in fruit cracking occurs directly across the fruit skin. Techniques currently employed include various spray applications of minerals, hormones, anti-transpirants and surfactants, in an attempt to reduce water uptake through the fruit cuticle. Helicopters are sometimes employed, at great expense, to create air flow around the trees in order to promote evaporation of fruit surface water. Rain covers, which prevent rain water from contacting and then pooling on the fruit, have been promoted and installed in local and overseas orchards, but with inconsistent results. No techniques addressing the alternative theory that water uptake through the internal tree system causes cracking have been developed. This is not surprising because there is, as yet, no direct evidence to support the theory fully.

Scope of the Project

Due to the expansion of the cherry industry in Tasmania and the need to supply export contracts in a reliable manner to maintain market share, managing the risk of rain-induced fruit cracking is paramount. There is little scientific data regarding the performance of cherry varieties in southern regions, in particular Tasmania, and inconclusive results indicating how trees interact with the local climate in terms of cracking. Therefore, based on the suggestions that cracking may be influenced by whole tree water relations (Sekse *et al.* 2005), an opportunity exists to not only investigate this alternative theory behind rain-induced cherry cracking, but also to assess the performance of varieties grown in Tasmania. The objective of this project was to increase the understanding of the mechanism/s involved in rain-induced fruit cracking, and determine the seasonal and varietal factors contributing to cracking. In this way, the project aims to provide a base of knowledge on which to develop management strategies and improve control of the disorder.

The project was undertaken over three seasons (2005/06 to 2007/08) with field trials running from full bloom, through all stages of cherry development until commercial harvest maturity. All trial sites were in southern Tasmania, within an hour's drive of Hobart. Most trials were undertaken on a commercial orchard at Grove, with a few select trials on orchards in Bushy Park and Campania and the Grove Research Station. Laboratory tests were undertaken at the School of Agricultural Science, University of Tasmania in Hobart, Tasmania.

Research Objectives

The research objectives of the project were as follows;

1. To determine the contribution of variety and season to cracking incidence
2. To assess the effect of rainfall levels, distribution and timing on cracking incidence
3. To investigate the development of different crack types
4. To determine the importance of whole tree water relations (the alternative theory) to crack development
5. To elucidate the diurnal fluctuations in fruit and leaf water relations
6. To examine the relationships of fruit and skin properties with cracking incidence
7. To examine the relationship of crop load with cracking incidence

Chapter 2

Literature Review

Introduction

Rain induced sweet cherry fruit cracking, by the simplest definition, is the rupture of the fruit skin and flesh in association with rainfall. Although the causes and mechanisms behind this process have been investigated for at least 75 years (Sawada 1931, Kertesz and Nebel 1935) they have not yet been fully explained. It is generally conceded that the process of fruit cracking is complicated and dependant on a wide variety of different factors (Sekse *et al.* 2005).

Two influential reviews of the sweet cherry cracking phenomenon have been published; a review paper by Sekse (1995a) (Sekse 1995b) and a chapter in “Cherries: Crop physiology, production and uses.” by Christensen (1996). A further review was presented by Sekse (2005) at the 4th International Cherry Symposium (USA, 2001). Sekse (1995a) (Sekse 1995b) proposed a build up of turgor within the fruit as the driving force behind fruit cracking. This increased turgor was explained by water uptake across the fruit skin, through cuticular fractures or pores, leading to epidermal cell wall disintegration. Christensen (1996) supported the theory of water uptake through the fruit skin as the leading cause of cracking, but mentions that earlier research suggested a dual mode of uptake through the skin and through the root system. The „fruit skin’ theory has been predominant in studies since these reviews were published, with many studies focussing on fruit and skin properties (Knoche *et al.* 2000, Beyer *et al.* 2002b, Beyer *et al.* 2002c, Knoche and Peschel 2002, Knoche *et al.* 2002, Beyer *et al.* 2005, Knoche and Peschel 2006, Weichert and Knoche 2006b, Weichert and Knoche 2006a). By 2005, however, Sekse (2005) added that sap flow (water movement through the internal system of the tree) could also cause increasing

turgor that leads to fruit cracking. A further review in 2006 by Simon (2006) mirrors the theme of Christensen (1996), claiming that earlier research pointed to involvement of root uptake of water in cracking development, but that recent studies showed skin uptake of water was more likely to be the sole contributor of crack development. It is interesting to note that the review by Simon (2006) focuses on management strategies based around the skin uptake theory, and omits discussion of any studies by Sekse and colleagues post 1987. Hence, this warrants further integrated research to clarify fully the process of rain induced sweet cherry fruit cracking.

Cherry Cracking Defined

The description of fruit cracking has been refined over the years (Simon 2006), yet this still remains an area for further clarification. Cuticular fractures, undetectable by the naked eye, have been recorded microscopically (Glenn and Poovaiah 1989, Sekse 1995b, Sekse 1995a, Børve *et al.* 2000, Knoche *et al.* 2002, Hovland and Sekse 2003) but the influence such development has on true cracking incidence remains unclear. True cracking, that is economically significant, remains inexplicable despite years of research and refers to larger visible cracking.

Crack Types

Visible cuticular cracking commonly occurs as one of three crack types (stem-end, apical-end or side cracks). Two occur at either end of the cherry fruit, shallow cracks close to the point of stem attachment, or at the stylar scar or apical end of the fruit. These cracks are commonly referred to as „stem’ and „nose or apical’ cracks respectively (Figure 2 and Figure 3). Both occur in concentric rings around the stem or apical areas without extending into the bulk of the fruit. A third type, deep cracks originating from the cheeks and extending over the bulk of the fruit in any direction and often penetrating the entire fruit tissue to the pit, is the most damaging, both physically and economically. This type of crack is referred to as a „side’ crack (Figure 4). The development of these different crack types has not been fully explained as few studies (Christensen 1972, Christensen 1996, Simon 2006) even attempt to categorise cracks into particular types.



Figure 2 Stem-end crack in sweet cherry fruit.



Figure 3 Apical-end crack in sweet cherry fruit.



Figure 4 Side crack in sweet cherry fruit.

Measuring Cracking Incidence and Susceptibility

In-field cracking levels are usually visually counted. The cracking index is a measure of varietal cracking and is determined by laboratory methods, taking no account of the environmental conditions of the field. Developed in 1931, the method involves immersing cherry fruit in water under controlled temperature, and calculating the percentage of cracked fruit (Christensen 1972). It is the results of these tests that determine a variety as having low, medium or high susceptibility.

Although the method has been declared sound (Christensen 1972) there are a number of concerns when using this as a guide to planting choice. Primarily, this method is not representative of field conditions, thus does not provide a clear indication of varietal susceptibility to cracking *in situ* (Christensen 1996). The test is also unable to hold true for different regions and climates, and seasons. However, in a small trial in France, using „Regina’ and „Lapins’ these tests have shown a good correlation between field grown fruit and fruit grown under controlled conditions (Quero-García *et al.* 2009).

Timing of Cracking Susceptibility

The incidence of cracking is assumed to occur in association with rainfall events at, or near, harvest maturity of fruit. Generally, the cracking susceptible period for cherries is taken to be during late Stage III of development, during the ripening process (see below). The review articles by Sekse (1995a (Sekse 1995b); 2005) put the commencement of susceptibility at a few weeks prior to harvest. Christensen (1996) is slightly more ambiguous, claiming that while cracking susceptibility occurs late in Stage III, it is also dependant on the length of ripening as influenced by season. Usenik *et al.* (2005) and Simon (2006) are equally cautious, stating respectively that cracking occurs during the ripening process and that susceptibility increases with maturity.

Cracking and Fruit Development

The growth pattern for cherry fruit consists of three distinct growth stages, two stages of rapid growth with a lag phase in the middle in a double sigmoidal pattern (Hovland and Sekse 2004), as first described in 1934 by Tukey and Young (Knoche *et al.* 2004).

The length of the growth stages is determined partly by variety, but is influenced by seasonal conditions. Stage I occurs during the first three to five weeks of cherry development and consists mostly of rapid cell division. Stage II (the lag phase) covers a period of only one to two weeks and is characterized by embryo development (pit-hardening) with little change in total fruit mass. The last stage, Stage III continues until maturity, a period of about four to five weeks. It involves

rapid growth through cell expansion, mesocarp development, and a rapid increase in size and mass. The third stage is perhaps the most crucial for cracking because it involves rapid water uptake and growth (Knoche *et al.* 2004).

Water supply to the tree during stage I is particularly important, given that water not only limits the rate of growth but that water potential influences cell division (McIntyre 1997). Developmental changes in xylem and phloem flow rates to fruit are consequently important. Generally, flow rates to fruit have been shown to increase with development (Lang and Thorpe 1989). In the early stages of growth in apples, xylem and phloem flows have been found to provide an equal contribution to growth (Lang 1990). As development continues into the middle and latter stages, this trend changes in favour of phloem flow (Lang 1990). Phloem contribution also increases with fruit maturity in grapes (Choat *et al.* 2009). Flow pathways could also be important in turgor regulation in cherries.

Stage II heralds the cessation of cell division allowing time for embryo development. During Stage III, in which fruit size increases through cell expansion, water again becomes crucial, particularly with regard to cracking and the pathways available for excess water uptake. Maximum fruit surface area expansion occurs during Stage III; at 53 days after full bloom (DAFB) (Knoche *et al.* 2001), with maximum weight increase between 53 and 59 DAFB (Hovland and Sekse 2004). Late stage III involves rapid cell expansion (with a decrease in intercellular spaces) and increased size and surface area of the cherry fruit. However, unlike other fruit (eg. grapes and tomatoes) there is no net increase in cuticular membranes or waxes (Hovland and Sekse 2004, Peschel *et al.* 2003), implying that cuticular covering per unit area dramatically decreases as the fruit

matures. Water uptake across the fruit skin is affected in Stage III, as fruit cuticular membrane conductance changes (Hovland and Sekse 2004). The cuticle provides diffusion resistance and leaching prevention but is not as efficient in this role as the cuticle of other fruits such as apples, pears and grapes (Sekse 1995b); (Hovland and Sekse 2004). Peschel *et al.* (2007) suggest that during maturation the changes in cuticle composition will affect transport barrier properties, wetting characteristics and mechanical properties of the membrane.

Soft fruits with thin cuticles, such as cherry fruit, can lose sugars, minerals and anthocyanins during rainfall events (Lang and Thorpe 1989). Firmness is claimed to be associated with cracking (Christensen 1996). Softening in sweet cherry occurs during ripening, and is due to changes in the pectin rich cell walls however, the changes reported in the activity of pectolytic enzymes did not correlate to changes in firmness in „Burlat’ cherries (Remon *et al.* 2006), supporting claims that more than one process is involved in softening (Brummell 2006). It has also been suggested that calcium plays a role in delaying fruit softening as the fruit approaches harvest maturity at the end of Stage III (Fallahi *et al.* 1997).

Levels of sugars and anthocyanins are important quality attributes of harvest mature fruit. In cherries, the strongest fruit sink strength occurs in Stage III, with spur leaves, shoot leaves and new leaves on current seasons growth all contributing to photoassimilation (Ayala and Lang 2008). In young stone fruit trees, under conditions that favour vegetative growth, secondary wood thickening provides the most preferential assimilate sink (Costes *et al.* 2000).

Harvest maturity of sweet cherry fruit has traditionally been difficult to assess accurately. A combination of colour and size are the major maturity indices used in the industry. Sugars (brix levels) are used occasionally but there is no desired set level to indicate maturity. Proteins have been identified as having potential to pinpoint maturity for a number of stone fruits (plums, peaches and nectarines), as they have been found to be synthesised only a few days prior to optimum harvest date (Abdi *et al.* 2002). No such test exists for cherries, but such tests would be very useful in terms of deciding harvest dates in reference to upcoming rainfall events and risk management. To date, the primary quality attributes for cherries have been size and colour, with firmness and lack of wound sites, including cracks, important for transport and shelf life. To the best knowledge of the author, no studies can confirm the exact time of day when cracking occurs. Anecdotal evidence is vastly different and contradictory.

Cherry Cracking Research

The most widely accepted and explored theory of cracking is that after rainfall an excess of water enters the fruit via direct movement across the fruit skin. This principally occurs during Stage III of fruit growth. As early as 1931 Sawada (1931) concluded that the cause of cracking is directly attributable to the effect of rain water on the cherry fruit surface in a localised manner, and that osmotic uptake is the driving force for water movement.

The review of cracking by Sekse (1995) concluded that water uptake occurred through fruit cuticular fractures or pores. This in turn leads to an increased turgor acting from within the fruit, sufficient to cause cracking. Reviews by Christensen (1996) and Simon (2006) both concur with this theory, and further suggest that this direct uptake is localised, occurring only at wetted parts of the fruit.

A diverse range of factors, and how they impact on driving water movement across the skin surface, have been investigated over many years. It is important to remember that studies are usually conducted on detached fruit in a laboratory setting.

Hydraulic Properties of the Fruit Skin and Cherry Cracking

Given the acceptance of the theory that water uptake across the fruit skin results in fruit cracking, it is of little surprise that this area of research has received so much attention.

Skin Properties

The cherry fruit skin is composed of a thin cuticle (1 μm) and up to eight dermal layers, the cuticle and dermis measuring up to 4.5 μm in thickness (Bargel *et al.* 2004). The cuticle is made up of hydrophobic cutin, with intrusions of wax films, an internal layer of hydrophilic cutin (with polyurinatedes and μ -1, L-glucans), with single cellulose microfibrils near to the epidermal layer. The cherry fruit has few stomata (85-200/cm²), compared with leaves (5 000-10 000/cm²) (Glenn and Poovaiah 1989) and compared with other types of fruit such as tomatoes (Gay and Hurd 1975). Cherry fruit do, however, contain pores that are permeable to polar compounds, such as water (Weichert and Knoche 2006b). The dermal layers of the skin consist of a single epidermis with regular, rectangular cells. Next to this are up to seven dermal layers of larger and irregularly shaped cells and pulp. These are composed of parenchymous tissue, and make up the bulk of the fruit (Sekse 1995b).

Skin Properties and Cracking

No relationship between epidermal cell size and other anatomical or structural features with cracking incidence had been found prior to Christensen's review in 1996. The vertical and lateral lengths of epidermal cells related strongly to

cracking susceptibility in a study in 2002 (Yamaguchi *et al.* 2002), but this was not mentioned in the review by Simon in 2006. In addition, osmolarity of the skin as a driving force for water was not found to be a significant factor separating susceptible and resistant varieties (Moing *et al.* 2004). It was suggested that rate of water uptake through the skin was more critical to cracking susceptibility than the total water content (Cline *et al.* 1995b). Water uptake across the fruit skin has been shown to be linear with time but no threshold value for rate of uptake has been determined (Christensen 1996).

Cuticle Properties

The cuticle has been shown to be a potential route for water uptake (Knoche and Peschel 2002), and is seen to be the first barrier or resistance to water flow (Huang *et al.* 2004). Thus, cuticle thickness, conductance and points of uptake have all been investigated. The ability of fruit to both gain and lose water (Beyer *et al.* 2005) has been posited as a concern because it will weaken the cuticle and predisposing the fruit to cracking (Kozlowski 1968, Yao *et al.* 2000). Exposed fruit were found to have a more inelastic cuticle according to Yao *et al.* (2000). This did not expand with increased water uptake, and was therefore considered to be more susceptible to cracking. Shaded fruit (capsicums, apples, tomatoes, cherries) have been reported not to experience as high a level of cracking as exposed fruit. This is likely due to the decreased exposure to direct irradiance, and thereby to a lower temperature, so enabling the cuticular structure to be maintained (Moreshet *et al.* 1999, Yao *et al.* 2000).

The composition of the cuticle of cherry fruit has been determined. More relevant to potential crack development is that while cuticular mass increased up to 36 DAFB, it thereafter decreased on a whole fruit basis. This resulted in a lower cuticular mass per unit area and decreased thickness (Peschel *et al.* 2003). Additionally, in a study by Knoche *et al.* (2001), cuticular membrane mass, per unit area, decreased after 31 DAFB. However, thickness of the cuticle did not explain resistance to cracking in a study of low, medium and highly susceptible cherry varieties in a study by Lane (2000), but did explain resistance to cracking in an earlier study (Belmans *et al.* 1990) and a later study (Demirsoy and Demirsoy 2004), as well as in cherry tomato fruit (Matas *et al.* 2004). In developing a model of fruit surface conductance, it has been concluded that total fruit surface conductance decreases with fruit expansion (Gibert *et al.* 2005). Fruit firmness is compromised in fruit with ineffective cuticular protection, but it has also been suggested that due to the rigidity of cuticular structures, cuticular fracturing was increased with irregular water supply (Sekse 1995a).

Cuticular Conductance

Cuticular membrane formation was reported to cease between 22 to 43 DAFB, and thereafter, as fruit size and surface area increased, conductance of the cuticle was found to increase by 108% (Knoche *et al.* 2002). Cuticular contribution to total conductance was found to be variable during rapid growth phases (Gibert *et al.* 2005), such as during Stage III of cherry fruit growth when fruit are susceptible to cracking. Stripping the cuticle was found to increase fruit surface conductance in mature cherry fruit (Knoche *et al.* 2000). The model proposed by Gibert and colleagues (Gibert *et al.* 2005) also claimed a decrease in stomatal

contribution to conductance with rapid fruit growth, from 80% to 20%. This is in direct agreement with Knoche *et al.* (2001) who reported a decrease in stomatal density from 0.8 mm² to 0.2 mm² over Stage III of cherry fruit growth. Furthermore, a positive relationship between stomatal density and liquid water conductance was established by Knoche *et al.* (2000), and Peschel *et al.* (2003) observed cherry fruit stomata to be non-functional at maturity. Glenn and Pooviah (1989) earlier determined that a cuticular covering existed over the guard cells and inner walls of stomata, and that water damage was not evident at or near the stomata of immersed fruit.

Preferential Pathways for Water Uptake

Other preferential pathways or locations for water conductance across the fruit cuticle and skin have been investigated. It has been suggested that fruit with cuticular fractures developed more visible cracks when immersed in water (Hovland and Sekse 2003), and that these cuticular fractures may account for the higher water absorption rates observed (Glenn and Poovaiah 1989) resulting in crack formation. It was further postulated that the stylar scar area may offer less resistance because there is an absence of cuticular membrane in this area (Glenn and Poovaiah 1989). The cuticular membrane at the stylar scar was shown to have a higher conductance than other fruit areas (Knoche *et al.* 2000) and conductance of the stylar scar area was found to increase with fruit size (Knoche *et al.* 2002). Furthermore, the stylar scar area was also shown to have a higher stomatal density than other areas of the fruit (Peschel *et al.* 2003). However, Knoche and Peschel (2002) and Beyer *et al.* (2002b), both claim that the stylar scar is not a preferential uptake point, as sealing of this area did not affect water uptake. These authors also

showed that the cuticle, cracks and the pedicel/fruit junction are preferential uptake points (Beyer *et al.* 2002b, Knoche and Peschel 2002). The stem cavity region, in which the pedicel/fruit junction lies, is astomatous (Peschel *et al.* 2003) bringing into question the exact influence of stomata in crack development.

These findings, although supporting the theory that water conductance of the cuticular membrane outweighs that of stomata, provide inconsistency in practical interpretation. The astomatous stem end area found by Peschel *et al.* (2003) contains a region of preferential uptake (Beyer *et al.* 2002b, Knoche and Peschel 2002), and the high stomatal density stylar scar (apical) end area (Peschel *et al.* 2003) is not supported as a preferential uptake point by two studies (Beyer *et al.* 2002b, Knoche and Peschel 2002). Yet a positive relationship between stomatal density and conductance has been claimed (Knoche *et al.* 2000).

More recent studies have found that cuticular conductance also outweighs that of either diffusion or transpiration (Beyer *et al.* 2005), that polar pathways exist in cherry fruit exocarp, and that pH affects the conductance of the cherry fruit surface (Weichert and Knoche 2006b, Weichert and Knoche 2006a). These pathways allow water uptake by viscous flow, and the permeability of these pathways to water and other polar substances can potentially be decreased through the formation of ferric precipitates (Weichert and Knoche 2006a). Potential benefits of this approach include the lack of resolubilisation with subsequent rain events, and therefore the need for one application only. However, the authors concede that ferric salts are not a practical solution for management, and that the pedicel/fruit junction remains unaffected.

Fruit Dimension and Cherry Cracking

Apart from fruit skin properties, other whole fruit features have been suggested to affect the incidence of rain induced cracking in sweet cherry fruit by changing the water uptake potential of the fruit surface (Christensen 1996). Factors such as size (Christensen 1975, Knoche *et al.* 2002)), shape (Sawada 1934), sugar levels (Moing *et al.* 2004), osmotic concentration (Sawada 1931, Moing *et al.* 2004) and firmness (Christensen 1975) have all been considered. These studies have also yielded inconsistent and contradictory results.

Although fruit size is generally assumed to influence the incidence of cracking (Christensen 1996, Simon 2006) it was shown in 1975 that size is only influential within varieties (Christensen 1975). Fruit shape is also assumed to influence the incidence of cracking (Christensen 1996). Furthermore, it has been claimed that fruit shape influences not only the incidence of cracking, but also the type of crack experienced (Sawada 1934). Indeed, the conclusion that cracking occurs at right angles to the curvature of the fruit (Sawada 1934) has not yet been disputed. A study categorising 24 cherry varieties by principle component analysis into similar shape groupings has been undertaken by Beyer and colleagues (Beyer *et al.* 2002a). These groups were not compared with known cracking indices for these varieties. Using the indices provided by Christensen (1975), a cursory glance seems to show no apparent trend implying any relationship between the indices (Christensen 1975) and shape (Beyer *et al.* 2002a). Beyer *et al.* (2005) and Simon (2006) both intimate that cherry shape will potentially influence cracking by conceding that water will pool in either the stem cavity or the apical depression

of an individual fruit after rainfall. This may explain earlier inconsistencies in areas of preferential uptake, and may depend on the varieties investigated.

Fruit with higher sugar levels or other solutes are considered more susceptible to cracking (Christensen 1996, Richardson 1998) , because the increased osmotic potential provides a driving force behind water uptake and movement across the fruit skin. However, not all studies support this statement. Moing *et al.* (2004) found no difference in skin or flesh osmolarity between susceptible and resistant varieties. Also, despite claims that high fruit sugar concentrations are associated with a higher incidence of cracking, this may not be a direct effect. Sugar concentrations increase with maturity, along with cell wall softening, at the time when fruit become more susceptible to cracking. Sekse (1995b) suggested that cracking as a result of surface water uptake is compounded by the softening of tissue from surface moisture. Nonetheless, it must be remembered that natural tissue softening occurs as a normal physiological change during ripening.

Based on ideas in these papers, fruit firmness has logically been suggested as influencing fruit cracking (Christensen 1996). Indeed Kertesz and Nebel (1935) claimed cracking was more evident in firm varieties and Simon (2006) claims the influence of firmness on cracking is an accepted opinion. However, no recent supporting evidence has emerged.

Tissue Mechanical Properties and Cherry Cracking

An increased understanding of the underlying mechanical principles involved in fruit cracking resulting from water uptake across the fruit skin would be advantageous in developing management strategies. Generally mechanical properties of fruit tissues are not easy to assess due to their inherently soft nature, and the requirement to use excised tissue. Mechanical properties are also described in several different ways; stress (force/area), strength (stress at tissue failure), strain (change in length/original length) and stiffness (stress/strain) (Vincent 1990), and elasticity and plasticity (reversible or irreversible deformation) (Christensen 1996).

Cherries, being a soft fruit, rely primarily on the mechanical properties of the skin to avoid rupture. In tomatoes, the cuticle has been identified as providing a substantial contribution to the mechanical properties of the whole fruit (Edelman *et al.* 2005). In cherry fruit the cessation of cuticle formation induced the onset of elastic strain on the cuticle (Knoche *et al.* 2004). Skin elasticity tests on loose detached skin have been described as unreliable as elasticity is dependent on tissue hydration (Rootsi 1960) and thickness (Cosgrove 1993). Edelman (2005) demonstrated that elasticity and plasticity were affected not only by hydration, but also by temperature. Hydration and temperature will change diurnally in the field, suggesting that time of harvesting and sampling will impact on the determination of mechanical properties in laboratory tests. Christensen (1996) called for improved measures of assessing mechanical properties, especially in the light of measurements being variable in different planes. Bargel (2004) supported this need for better assessment tools by investigating a new two dimensional tension

test for cherry skin. Natural variation also occurs between varieties; a varietal difference in stress and strain of fruit skin has been demonstrated (Zoffoli and Jauregui 2009). The stress experienced by the whole fruit before rupture occurs is also dependent on the internal turgor pressure. Methods for determining critical turgor pressure in excised grapes have been developed (Considine and Kriedemann 1972), and for the resultant tangential stress experienced by the skin (Considine *et al.* 1974). The tangential stress can be determined by considering the size of the fruit, the thickness of the skin and the internal pressure. Given that cracking in cherries can be deep, it is necessary to consider the mechanical properties of the flesh or pulp of the fruit along with skin properties.

Current Management Strategies

The current available management strategies are all based on minimising water uptake across the fruit surface, by either complete exclusion (harvesting fruit prior to rainfall and/or using rain covers) or providing a resistance of some kind (spray applications). Other strategies include the choice of variety and rootstock, as it is believed that some varieties are inherently more susceptible than others (Simon 2006). Variety selection is based on a cracking index. This gives a rating of cracking after detached fruit have been fully immersed in water, thus replicating surface water exposure. It is not a field based representation of cracking, and does not consider the situation of partial surface wetting.

Harvest Timing

Timing of harvest is primarily aimed at fruit maturity. However both anecdotal and published evidence (Usenik *et al.* 2005, Simon 2006) suggests that growers consider timing of harvest as a cracking management strategy. It appears to be common practice for some growers to harvest slightly immature fruit when there is a high risk of rainfall occurring at harvest maturity, despite evidence that late harvest produces fruit with the most appealing taste, texture and nutritional value (Diaz-Mula *et al.* 2009).

Covers

Large covers that protect the whole tree from rain have been designed to avoid accumulation of water on the fruit surface. There are many types, but generally covers are made from cross laminated, UV stabilized polyethylene. These can be individual umbrellas supported by poles, or can extend the length of the row on wire frames with steel or wooden supports. They were first used in Europe. In the southern hemisphere, uptake of this technology predominantly occurs in New Zealand where there is a comparatively larger expenditure on risk management for high value fruit for export to Japan (Cline *et al.* 1995a, Webster and Cline 1994). For permanent structures, growth retardants or dwarfing rootstocks may need to be used to limit the height of canopies, and in dry years the cost may outweigh the benefits (Webster and Cline 1994).

Covers have shown to have variable effects on incidence of cracking. Results from Norway showed a 10% decrease in cracking compared with uncovered fruit, when covers were applied at the onset of growth Stage III (Cline *et al.* 1995a). Cline *et al.* (1995a) claimed that the reduction in fruit cracking (using varieties „Ulster’, ‘Sam’ and „Van’) in covered trees was dependant on rootstock, suggesting that other factors exert a greater influence on susceptibility. They further concluded that the cracking index of covered fruit was higher than uncovered. Wermund *et al.* (2005) reported that both „Colney’ and „Van’ fruit under covers achieved a higher cracking index than those uncovered. No significant differences were recorded in fruit properties due to covering; the explanations provided claimed that due to the set up of covers in late Stage III of fruit development, there would be little influence on cell division and hence final

size (Cline *et al.* 1995a). Børve *et al* (2003) claimed that covers of three different types had no effect on fruit quality parameters such as firmness and weight. Only umbrella covers were claimed to negatively affect soluble solids, colour and ripeness (Børve *et al.* 2003).

Even with excellent cover from rain, cracking still occurs (Cline *et al.* 1995b, Webster and Cline 1994), suggesting another mechanism or a combination of mechanisms are involved in inducing cherry fruit to crack in addition to direct water entry across the fruit skin. It was also observed that temperature and humidity was increased under covers, posing a greater threat of disease (Cline *et al.* 1995b, Simon 2006), although this was not reported in the study by Børve *et al* (2003).

Sprays

Hormones

Gibberellic Acid (GA3), a plant growth hormone, is routinely used in commercial cherry production to increase fruit size and firmness, reduce surface marking and delay ripening. It is generally applied at 15 – 30 ppm, 3 – 4 weeks prior to commercial harvest (Looney and Lidster 1980, Weaver 2005).

The effect of GA3 applications on fruit cracking has been documented. Webster and Cline (1994) showed that there was no effect of GA3 application on cracking when the fruit surface was wetted from rain, and that the occurrence of cracked fruit increased if fruit remained wet for four hours after application. Other studies in Belgium revealed that GA3 treated fruit had thickened cuticles, but a similar level of cracking to untreated fruit (Webster and Cline 1994). In contrast, research in Oregon found that GA3 promoted large side cracks in fruit, but reduced the occurrence of stem cracks (Webster and Cline 1994). Usenik *et al.* (2005) found an increase in the cracking index for GA3 treated fruit in only one out of three varieties but also noted that, across varieties, there was an increase in water uptake of GA3 treated fruit.

Paclobutrazol, a substance that prevents the synthesis of GA, has been found to increase calcium levels (see below) in fruit and shorten internodes (Webster and Cline 1994), with the implication that fruit firmness increased, and resultant increased leaf coverage acted as protection from rainfall. The same authors found that application of GA3 did not affect the total or soluble levels of fruit calcium. No effect on fruit firmness from either soil or foliar applied paclobutrazol was

seen in a study by Looney and McKellar (1987). However, when used in conjunction with GA3 it was shown to increase fruit size, increase stem length, decrease soluble solids and delay colour development (Looney and McKellar 1987, Webster and Cline 1994).

The effects of naphthalene acetic acid (NAA) application have been investigated in the United States of America and Spain where it was found to reduce water uptake through the fruit surface. When applied to fruiting trees at a rate of 1mg/L, thirty to thirty five days prior to harvest, NAA was found to reduce cracking incidence, however, when applied at the same rate four to eight days prior to harvest the incidence of cracking increased (Webster and Cline 1994). Unfortunately these results have not been reproduced elsewhere despite several attempts (Webster and Cline 1994).

Overall, the effects of hormone application to cherries with the aim of reducing cracking incidence have yielded inconsistent and sometimes contradictory results. Nonetheless, studies have been conducted on different varieties, in different regions, and given that hormone application has been part of normal orchard practice, continued investigation is warranted.

Minerals

The most common mineral employed in the management of fruit cracking has been calcium chloride (CaCl_2), because calcium would increase bond strength of cell walls, increase epidermal thickness and alter cuticular membranes (Marschner 1995). These factors would, in turn, reduce the hydraulic permeability of cell

membranes, thereby decreasing water uptake through the skin of the fruit (Glenn and Poovaiah 1989, Simon 2006). This all depends on calcium being available to the fruit, and as such, is highly affected by water supply and fruit maturity (Landsberg and Jones 1981, Schlegel and Schönherr 2002). Calcium is also a xylem mobile mineral, and fruit xylem connections and pathways are reduced during maturation (Kozłowski 1968, Huang *et al.* 2008), thus early accumulation is vital.

Applications of calcium chloride via overhead irrigation after rainfall events during the more cracking susceptible Stage III of cherry growth has had some success, but again results are inconsistent (Grubich 1998, Lang *et al.* 1998). Calcium applications in conjunction with copper were more effective in reducing cracking in cherries than calcium alone (Brown *et al.* 1995). Tomato fruit cracking showed a slight decline in early and weekly calcium applications (Huang and Snapp 2004). This early and repeated spray pattern for calcium uptake is supported by studies in apples (Schlegel and Schönherr 2002) as well as cherries (Fernandez and Flore 1998)

Calcium penetration rates have been found to decrease with maturity in several varieties of apples (Schlegel and Schönherr 2002). Lidster *et al.* (1979) reported enhanced calcium uptake rates in the sweet cherry variety „Van’ with the use of thickeners and surfactants. The stylar end of cherry fruit has been identified as the preferential site of calcium uptake in variety „Bing’ (Glenn and Poovaiah 1989). It has been suggested that calcium uptake across any fruit surface is inhibited by cuticle layers and low density of functional stomata, and that calcium applications had no significant effect on cracking incidence in litchi (Huang *et al.* 2008).

Instead cracked litchi fruit had significantly higher levels of structural calcium. It was therefore concluded that structural calcium in the pericarp in litchi was an integral component in fruit cracking, but this was not influenced by late calcium spray applications (Huang *et al.* 2008).

In addition to calcium uptake, application of calcium to the fruit surface was also thought to reduce water uptake through the skin due to the osmotic potential of the calcium residue on the surface, thereby decreasing the potential gradient across the fruit skin (Lang *et al.* 1998, Wermund *et al.* 2005, Simon 2006). These beneficial effects have not been fully supported in the literature and the numerous spray trials have yielded inconsistent results (Hanson and Proebsting 1996, Webster and Cline 1994). Calcium treatments did not significantly affect the rate of water uptake through skin in detached „Bing’ fruit immersed in solution (Glenn and Poovaiah 1989), yet it reduced water uptake in tomatoes (Huang and Snapp 2004).

A major limitation of using direct calcium application to prevent cracking is the unsightly residue left on the fruit (Simon 2006). Aluminium and boron sprays have been trialled in the United States of America, again with inconsistent results and residue deposits (Webster and Cline 1994). Simon (2006) cited one early study (1949) using boron spray as being effective on one variety only. In Poland, an extensive study of boron nutrition showed no affect of soil or foliar applied boron on the incidence of cracking (Wojcik and Wojcik 2006). No other mineral salts have been found to be effective in reducing cracking (Simon 2006).

Antitranspirants

Antitranspirants, such as Bioguard® (a calcium based product), and Vapor Gard® (a terpene polymer providing a protective film) have been trialled in relation to fruit cracking, on the premise that they will limit water uptake as well as loss from the fruit (Hanson and Proebsting 1996). Antitranspirants are commonly used to reduce plant water stress (Landsberg and Jones 1981), however they have also been shown to negatively impact on the levels of soluble solids because they limit gaseous exchange (Webster and Cline 1994). Results have been inconsistent and unable to be reliably reproduced (Richardson 1998). Additionally, such applications have a tendency to leave surface residue (Webster and Cline 1994). Nonetheless, Bioguard® reduced cracking by 52% in variety „Van’ in trials in South America (Torres *et al.* 2009). Recent investigations of a copolymer of stearic acid, cellulose and calcium (SureSeal), has shown that antitranspirants may have some potential. A preliminary trial in Norway resulted in a 15% reduction of cracked fruit with the application of SureSeal in conjunction with rain covers (Long *et al.* 2009).

Surfactants

Surfactants, when applied to fruiting cherry trees, have shown similar undesirable effects to antitranspirants, with the added downfall of being easily washed off by rain. Some promising results were shown in Australia where reduction in cracking was achieved in some trial seasons, but this was not consistent (Granger and Traeger 2002). In Germany, cracking incidence was reduced by 50% when applied one week prior to harvest (Webster and Cline 1994). However limited trials have been undertaken and the potential effects remain unclear.

Variety

As yet, no one variety has successfully been shown to be completely resistant to fruit cracking. Choice of resistant varieties has largely been subjective, due to a lack of supporting literature (Christensen 1996) and the existence of a cracking index that cannot effectively be used for different regions (Wermund *et al.* 2005).

Generally, varietal cracking susceptibility is discussed in terms of being low, medium and high, but even these categories can vary considerably by region and season. In 1934, Kertesz and Nebel conceded that to correlate cracking with varietal morphological features would be hazardous. This statement was confirmed in 2000, when a study investigated and found no effect of fruit properties of low, medium and high susceptibility varieties of sweet cherry fruit (Lane *et al.* 2000). No single character determines the stress resistance associated with cracking resistance in grape varieties, but it has been suggested that this is due to a combination of geometric, structural and physiological factors (Considine and Brown 1981). That no true variety susceptibility test exists for cherries to date supports the claim that more than one factor is involved. Varietal factors, other than morphological features, considered with regard to cracking have included sugar levels, firmness, water relations and rheological properties (Kertesz and Nebel 1935, Christensen 1972, Cline *et al.* 1995a, Beyer and Knoche 2002, Yamaguchi *et al.* 2002).

Varietal differences influencing planting choice are therefore mainly based on size, colour, firmness, maturation dates and consumer preference. The choice of

rootstock used has traditionally been limited to availability, suitability to production systems and site selection. Only more recently has the effect of rootstock on cracking has been investigated (Cline *et al.* 1995a, Hovland and Sekse 2003). In a trial examining the effect of rain covers and rootstocks, fruit grown on rootstock F:12/1 showed no significant decrease in cracking, covered or uncovered. In comparison, fruit grown on rootstock „Colt’ showed a significant decrease in field cracking both covered and uncovered (63% and 5% respectively) (Cline *et al.* 1995a). Hovland and Sekse (2003) showed that rootstock influenced the development of cuticular fractures, and cracking incidence when immersed in water and Simon (2006) commented that rootstock influenced cracking after immersion.

Summary

Strong progress has been made in understanding the role of the fruit skin in crack development in sweet cherry fruit; some studies confirming the theory of direct water uptake across the skin results in cracking. Despite extensive studies, there is still some uncertainty surrounding the role of the skin and cuticle in water conductance and its consequent influence on cracking. The range of methods and varieties used are extensive, and it must also be remembered that responses of detached fruit will not necessarily be representative of fruit behaviour *in situ*.

Sweet cherry fruit cracking has inevitably been associated with rainfall, and as such, the management practices discussed have been developed on the basis that fruit exposed to rainfall was likely to crack. These practices were generally aimed at reducing the amount of rain on the fruit, or altering the fruit surface with various sprays to limit water uptake across the fruit skin. Trials have been undertaken in different locations, on a range of varieties and under different orchard management practices. Commercial uptake of strategies to manage cherry cracking has been slow, largely due to lack of reproducibility of research findings and the consequent uncertainty, and the significant financial investment required. For example, rain covers are capital intensive and calcium sprays reduce the aesthetic quality of fruit. That cracking still occurs under covers questions the widely accepted theory that cherry fruit splitting is induced only by water uptake across the fruit skin. It is likely that more than one mechanism for fruit cracking exists, as suggested by Sekse (2005).

Although Sekse's review in 1995 led to a spate of skin focussed research, Sekse (2005) added that sap import through the fruit stem is a major cause of turgor build up in the fruit and that the mechanisms driving water uptake that lead to cracking are complex and integrated. In spite of it being the „normal' pathway of water to the fruit, the whole tree hydraulic system and the impact of vascular movement of water into fruit in relation to crack development has received scant attention.

Kertesz and Nebel (1934) implicated internal vascular uptake in the development of fruit cracking many years ago, suggesting that swelling of cherry pulp occurring from normal fruit expansion could be a contributing factor. Vascular movement of water as an alternative mechanism driving cracking has also been suggested (Sekse 1995a). It has been established that cherry fruit will still crack under covers (where the fruit surface remains dry) and that cracking incidence can be dependent on rootstock (Cline *et al.* 1995b). Although limited in number, these findings clearly implicate root water uptake and movement via the vascular system in crack development.

In addition, papers investigating tissue structural failure in tomatoes (Ohta *et al.* 1998), capsicums (Aloni *et al.* 1999) and carrots (Gracie and Brown 2004) report or implicate the internal vascular system. Reduced cracking in tomatoes was recorded under high light intensity treatments due to increased stomatal opening, increased influx of solutes to the leaf and efflux of solutes from the fruit (Ohta *et al.* 1998). During periods of expansion in capsicum reduced cracking was also recorded in the absence of surface water, implying a growth mediated vascular process. Furthermore, it was hypothesised that vascular supply of photoassimilate

was linked to splitting disorder in carrots and leaf trimming could be a viable management option (Gracie and Brown 2004). Not only should this alternative water uptake pathway be explored, but in order to further develop effective management strategies for cherry cracking the relationship between water uptake pathways and environmental influences must also be explored.

Water relations in cherry fruit trees have been suggested as having an effect on the cracking of cherries and warrants inclusion in a review of literature. However, much of the research on whole plant water relations of trees to date has focussed on forestry plantation species as opposed to fruit bearing species, for the purpose of determining irrigation scheduling in some deciduous trees (Storey and Treeby 1999, Goldhamer and Fereres 2004). The effect of whole tree water status and the interaction with the environment on fruit quality (which includes cracking) has not been extensively explored in stone fruits, but the work of Tyree and associates indicates that this is a viable line of investigation (Tyree and Ewers 1991).

The ability of fruit to both gain and lose water is thought to influence cracking, by ultimately weakening the cuticle and predisposing the fruit to cracking (Kozlowski 1968, Yao *et al.* 2000). Diurnal patterns of fruit diameter shrinkage and expansion has been recorded in capsicums. (Aloni *et al.* 1999, Yao *et al.* 2000). Aloni *et al.* (1999) also claimed that the magnitude of the stress of repeated dehydration and rehydration influenced cracking susceptibility.

Cracking in the absence of surface water has been demonstrated for sweet cherries under covers (Cline *et al.* 1995a) and Aloni *et al.* (1999) found that cuticular cracking in bell peppers was enhanced by the addition of internal water. Internal,

or vascular, water flux after rainfall needs to be established and conditions creating preferential water flow to the fruit are of high importance. Furthermore, the potential for this flow to provide water sufficient to result in cracking needs to be established.

Research Impetus

Although decades of rain-induced sweet cherry fruit cracking research exists, the exact timing of true crack development, or of fruit susceptibility to cracking, is not yet known. Research to date has not yet provided definitive answers as to how, when or where visible cracking is first initiated on the fruit. The exact timing of cracking susceptibility is difficult to assess due to differing climatic conditions between both growing regions, and seasons. In addition, methods used to assess varietal susceptibility are not dependable; they are based on laboratory assessments and involve only one mode of water uptake (across the fruit skin), even though two modes (direct skin uptake and vascular uptake) have been suggested in the literature. Based on this review of literature surrounding cherry cracking, the question of vascular involvement appears to be insufficiently represented. A conclusive answer as to the involvement of the alternative water uptake pathway (through the vascular system) is needed in order for research into the mechanisms underlying sweet cherry fruit cracking to progress. The primary aim of this project to address this imbalance and by providing an answer to this question contribute to the future management of cherry fruit cracking. It is hypothesised that this alternative water pathway for excess water uptake by the fruit exists (See Research Objectives – Chapter 1).

Chapter 3

General Materials and Methods

Location of Field Trials

All research conducted throughout this project was undertaken in southern Tasmania, Australia, in three horticultural regions. All regions were located within 50km from Hobart (42°50'S, 147°21'E). The first region, the Huon Valley (42°98'S, 177°08'E) (Site 1) contained the primary trial site, a commercial orchard at Grove, and the nearby Grove Research Station. The second region, the Derwent Valley (42°71'S, 146°90'E) (Site 2), contained a commercial orchard at Bushy Park and the third region, the Coal River Valley (42°83'S, 147°48'E) (Site 3) contained a commercial orchard at Campania (Figure 5).

Southern Tasmania has a cool temperate climate and, compared with warmer regions found on mainland Australia, provides a relatively long growing season for cherries. All three regions experience mean monthly maximum temperatures of less than 25°C over the summer period. Annual mean rainfall (mm) is 743.1, 574.6 and 601.9 for Grove, Bushy Park and Campania respectively. Mean monthly rainfall (mm), and maximum and minimum temperatures (°C) for the three trial sites are given in Figure 6.

Trial sites in regions 1 and 2 were netted, while in region 3, the trial site was not netted. All sites in region 1 and 3 are located on brown sodosols and these sites use mounded soil rows with one line dripper irrigation systems. The site in region 2 is located on deep windblown sands with excellent drainage. This site uses micro sprinkler irrigation. All sites have an inter-row spacing of at least 2 m with between tree spacing of between 1.5 to 2.5 metres.



Figure 5 Map of Tasmania, Australia, showing cherry production regions, 1, 2 and 3, The Huon, Derwent and Coal River Valleys, with trial sites located within each region.

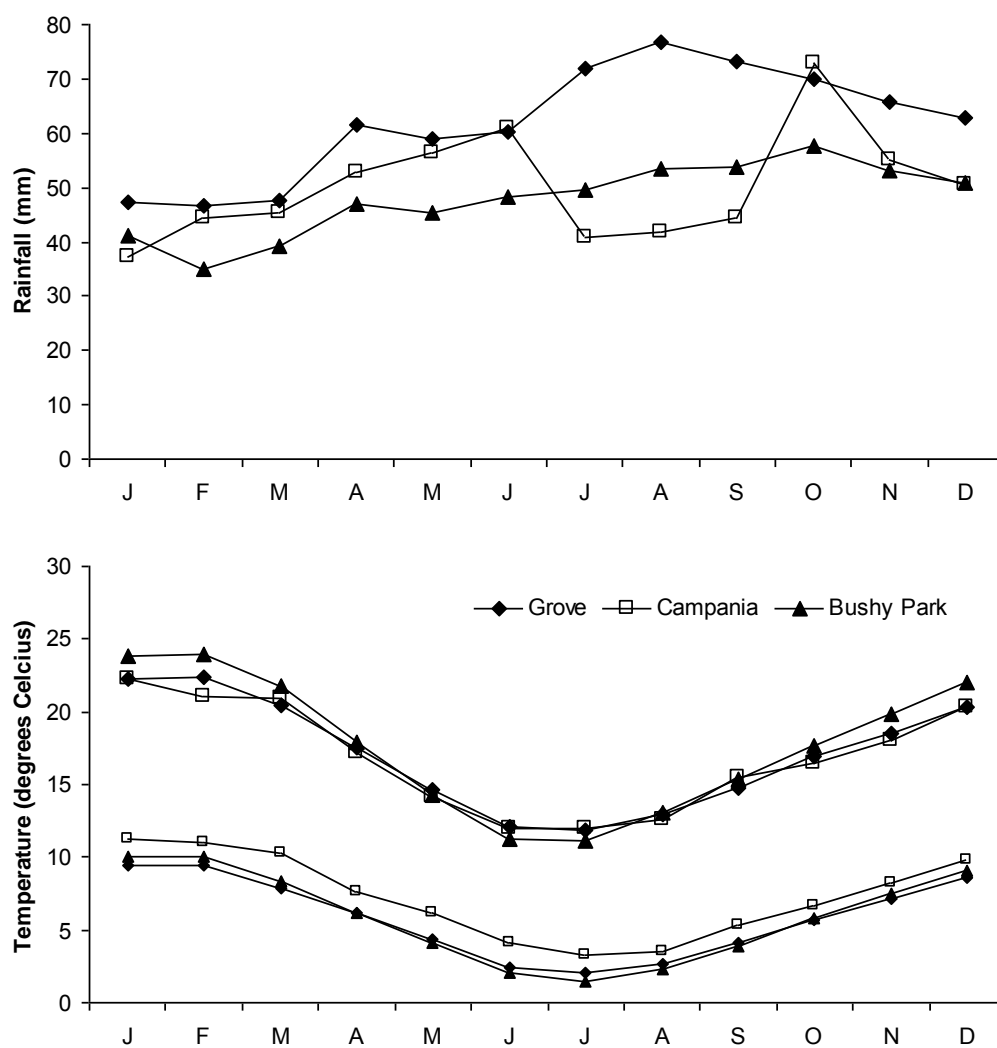


Figure 6 Mean monthly rainfall (mm) and maximum and minimum temperatures ($^{\circ}\text{C}$) for the three trial sites. (Mean is over last 50 years).

Timing of Field Trials

All field trials were designed around the growth stage of the fruit and undertaken between full bloom to commercial harvest. Growth stages are similar at all three regions, with commercial maturity for the same variety usually occurring first in region 3 (Coal River Valley) followed by regions 1 (Huon Valley), then 2 (Derwent Valley).

In the southern hemisphere growth cycle budburst occurs in mid September, full bloom is reached by early to mid October, and harvest occurs from late December through to early February. Leaf fall then occurs from late February through to March. Starting trials during full bloom was avoided in order to minimise damage to fruit and potential fruit set. The majority of field data collection occurred during the critical pre harvest period of three weeks prior to commercial harvest for each variety, so ranged from mid December to the end of January. Sampling of fruit occurred in line with commercial harvest dates for each variety in each year.

Tree Selection

Tree selection for field trials occurred in mid to late October, minimising disruption to fruit set. Trees were selected based on uniformity of size and health and marked with flagging tape. There were at least two buffer trees of the same variety between each selected treatment tree. For trials involving treatment application, treatments were randomly assigned to trees at this stage and marked with different coloured flagging tapes, or combinations of tapes. For all trees in all trials tree size was recorded as trunk circumference, measured in centimetres at a point 5 cm above the graft union. Trunk cross-sectional areas (TCSA) were calculated for each tree for the area (A) of a circle using the formula ($A = C^2/4\pi$) where C = circumference (cm). All trees were mature, and in their fourth leaf at the commencement of the project unless stated otherwise. Details of varieties available for each trial are given in relevant chapters.

Fruit Quality Measurements

Fruit Harvesting

Fruit was harvested on commercial harvest dates for each variety in each year. On harvesting days, picking occurred from 7 am to 12 noon, in line with standard commercial practice. All fruit on each tree was picked, even if it was damaged, with the stems attached as in commercial picking. Picked fruit were kept in the shade, and transported to refrigeration facilities on the same day. The total number of fruit for each tree was recorded as the number counted at harvest. In each season, a sub sample of at least 30 blemish-free fruit were randomly selected from fruit harvested from each variety to undertake fruit quality measurements (size, weight and total soluble solids) on the same day as harvest.

Fruit Size

Fruit size was measured as the diameter of the fruit (mm) using Vernier callipers. This was taken at the widest axis of the fruit, approximately two thirds from the apical end of the fruit (Figure 7).

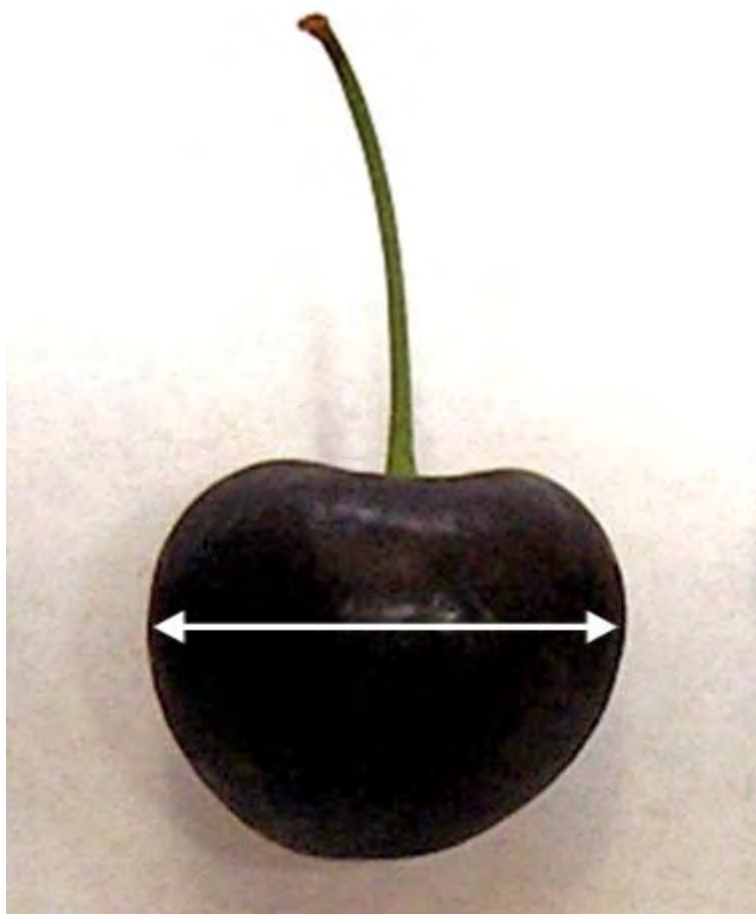


Figure 7 Measurement point for fruit diameter, indicated by white line, on a mature ‘Sylvia’ cherry.

Fruit Weight

Individual fruit weight with stem detached was recorded on Mettler Toledo scientific balance scales within four hours of being harvested.

Total Soluble Solids

Total soluble solid (TSS) concentration (brix^o) was assessed using a Shibuya Optical hand held refractometer using freshly expressed juice from individual fruit.

Crack Determination

The incidence of total cracking and crack type for each tree in each trial was determined on the same day as harvesting once fruit had been returned to the laboratory. The number of each crack type (as described in Chapter 1) was recorded. Cracked fruit required for further analysis was then sealed in plastic bags, labelled and frozen at -18°C.

Crop Load

After harvesting, total fruit, including damaged fruit, was counted and used to calculate crop load for individual trees. Crop load was expressed as total fruit number per trunk cross sectional area (TCSA).

Experimental Designs and Data Analysis

Field trials were designed as randomised complete blocks, unless otherwise stated. Application of treatments for each trial is described in research chapters. The number of replicates varied between trials but for field trials the minimum number was three.

Data analyses were performed using statistical software SPSS (Version 17.0) and SAS (Version 9.1). Specific tests are described in experimental chapters. All results quoted as „significant’ are at a probability level (P) of ≤ 0.05 . All graphs were generated using Microsoft Office Excel 2007.

Chapter 4

Incidence and Type of Cracking in Sweet Cherry (*Prunus avium* L.) are Affected by Genotype and Season.

This chapter covers an exploration of cracking and cracking patterns across varieties and seasons in order to assess the cracking response of Tasmanian cherry varieties to late season rainfall. It addresses the first two experimental objectives as follows, and indicates a difference in crack type development.

- To determine the contribution of variety and season to cracking incidence
- To assess the effect of rainfall levels, distribution and timing on cracking incidence

Introduction

Fruit cracking has been recorded in many sweet cherry (*Prunus avium* L.) varieties, with incidence as high as 63 % of harvestable yield (Cline *et al.* 1995a). A loss greater than 20 -30 % of yield has been considered uneconomical to harvest (Hanson and Proebsting 1996).

Given this commercial significance, management of cracking has received a great deal of attention with research ranging from practical strategies to investigations of the underlying mechanisms (Christensen 1996, Sekse 1995b). As yet, no cherry producing region has claimed to be immune to the cracking problem and, despite rain covers being employed, effective control remains expensive and unreliable. In Tasmania, reliable production of large, late season fruit remains a major challenge for the local industry. Fruit size is reportedly linked to incidence of fruit cracking, and cracking also coincides with rainfall (Cline *et al.* 1995a, Christensen 1996).

Three main crack types have been characterised (Christensen 1972). These largely refer to the position and shape of cracks; smaller circular cracks around the stem end (stem cracks) and the apical end (apical cracks) of the fruit and also larger, deep cracks forming over the cheek of the fruit in all directions (side cracks). However, variation in the proportion of these crack types (stem, apical or side) has received scant attention and the vast majority of field studies, in which cracking was measured, have recorded total cracking but have not differentiated between the different types.

Sekse (1995b) (Sekse 1995a) included a severity rating in order to further define cracking, but did not differentiate between the different types of cracks. Christensen (1972) suggested that time of maturity (early or late varieties) influenced crack type. It therefore remains unclear whether crack type varies between variety, seasonal conditions or is influenced by management.

In addition, current cracking indices to test cracking susceptibility (Cline *et al.* 1995a) are generally based only on laboratory immersion tests as described by Christensen (1972). These take no account of the external environment in which fruit are growing at the time of crack development. Lane *et al.* (2000) suggested that there is a strong environmental or seasonal influence on cracking susceptibility because fruit properties did not adequately explain differences in susceptibility.

Fruit properties such as fruit size, pulp osmotic potential and skin cuticular characteristics have been documented and investigated in much cherry cracking research (e.g. (Christensen 1972, Lane *et al.* 2000, Yamaguchi *et al.* 2002, Moing *et al.* 2004), but none have strongly correlated with levels of cracking or the occurrence of different crack types. A variety difference in the development of microscopic cuticular fractures in varieties ‘Van’ and ‘Sunburst’ was found by Hovland and Sekse (2003). The variety ‘Van’ has long been considered one of the most susceptible varieties in current use, and has been used as a control in comparative studies. However, Lane *et al.* (2000) found no difference in skin strength (tested by the force required to rupture the skin) between varieties of low, medium and high cracking susceptibilities, according to current cracking indices.

The present study aimed to determine whether there were any seasonal effects on the development of different crack types across a range of varieties grown commercially in Australia.

Materials and Methods

Plant Material

Sweet cherry trees, in their fourth leaf at commencement of trials, on F12/1 rootstock and pruned to a Spanish bush style in a commercial orchard located near Grove, Southern Tasmania (Australia) (42°98'S, 177°08'E) were used in all trials. Irrigation was applied by drippers morning and night (4 L/hr) during the entire growing season, and pest and disease control and nutrient management were as standard for the local industry. In each year, three trees of each variety were selected at random. Each of the selected trees was buffered on both sides (in the row) by trees of the same variety.

Trials were undertaken over the late November to late January (southern hemisphere) harvest seasons in 2004/05, 2005/06, 2006/07 and 2007/08. Although the nine varieties available for study included 'Lapins', 'Sylvia', 'Simone', 'Summit', 'Van', 'Sunburst', 'Kordia', 'Regina', 'Sweetheart', not all could be included each year due to various commercial considerations. Varieties 'Sylvia', 'Lapins' and 'Simone' were selected in all seasons, 'Sunburst' in 04/05, 'Summit' and 'Van' in 04/05, 06/07 and 'Kordia', 'Regina' and 'Sweetheart' in 05/06, 06/07.

Similar measurements were taken in all trials in each season. All fruit was harvested between 7 am and 12 noon on commercial harvest dates as determined by the grower according to commercial practices. Cracking assessments, morphological measurements and laboratory based measurements determining

critical turgor were undertaken on the same day as harvest. Blemish free fruit were separated from damaged fruit for each variety and a subsample of a hundred blemish free fruit (per variety) was selected at random, sealed in plastic bags, placed on ice and returned to the laboratory for immediate analysis. A further subsample of fifty fruit (per variety) was also selected at random, sealed in plastic bags, placed on ice and immediately returned to the laboratory and frozen.

In 2004/05, the measurement period was extended with fruit properties measured three times in the four weeks prior to harvest maturity on all available varieties. Two small trials to determine cracking distribution were also carried out on ‘Simone’ only. In the first of these, cracking was recorded separately on branches oriented closest to north and south. In the second, branches were divided into upper and lower regions, and sampled separately. In 2004/05 trials, cracking was assessed on the tree, and fruit that cracked prior to commercial harvest were marked with paint and fruit diameters were recorded *in situ* to avoid changes in fruit load prior to final harvest.

Climate data (daily minimum and maximum temperatures, rainfall in the 24 hours prior to 9 am, humidity, sunlight hours) for the months preceding harvest dates was obtained from the Australian Bureau of Meteorology Station at Grove (situated less than 1 km from all trial sites). For the effects of preharvest rain on cracking, the three weeks prior to commercial harvest were taken as the critical period in line with the 2004/05 data, grower observations and other cracking studies (Sekse 1995a, Lane *et al.* 2000).

Cracking and Fruit Morphology Measurements

Total fruit numbers were recorded for each tree and cracked fruit, divided into side, apical or stem cracks, were counted at harvest maturity. In the first year, weekly and cumulative total cracking and crack type percentages for each variety were calculated. At harvest in each season, individual fruit weight (g) and diameter (mm), measured as maximum diameter perpendicular to the stem/apical axis across the widest part of the fruit, were recorded.

Total soluble solids were measured as degrees Brix using a hand held refractometer. Randomly selected segments of diced fruit from frozen subsamples were placed in 2.5 ml eppendorf tubes and centrifuged at 10 286 g for five minutes, from which osmotic potential (π) was determined using a Wescor 5520 VAPRO® Vapor Pressure Osmometer.

Critical Turgor and Tangential Stress

In 2004/05 and 2006/07, pedicels were carefully removed and fruit was randomly allocated to five lots of ten fruit in 2004/05 and five lots of twenty fruit in 2006/07. Each lot was then immersed in one of five polyethylene glycol (PEG) solutions of known osmotic potential; 0, -0.25, -0.5, -1.0 and -1.5 MPa in 2004/05, and 0, -0.3, -0.6, -0.9 and -1.2 MPa in 2006/07. The osmotic potential solutions were formulated using PEG 6000 (Sigma-Aldrich) based on concentration (Michel and Kaufmann 1973), and confirmed using a Wescor 5520 VAPRO® Vapor Pressure Osmometer calibrated against NaCl standards. Distilled water was used for the 0 MPa solutions.

At regular intervals after immersion, fruit was visually assessed for crack development, and crack type was noted. No further cracking occurred after 44 hours in any of the solutions for any of the varieties tested. Thus it was assumed that the water potential of the fruit had equilibrated with the PEG solution. The relationship between water potential and percentage of fruit cracking after 44 hours was taken as the water potential at which 50 % of fruit had cracked (α) with 95 % confidence limits calculated using logistic regression (SAS version 9.1). The turgor pressure at which 50 % of the fruit cracked was then calculated as

$$CT_{50} = \alpha - \pi$$

Tangential stress (TS) experienced by each variety at CT_{50} was calculated using a formula for a thin walled sphere, similar to the approach employed by Considine and Kriedemann (1971) for grapes. Skin thickness of ten fruit for each of the varieties was recorded microscopically, and an average thickness used to calculate the tangential stress experienced by each variety was determined using the formula;

$$TS = (T \times r) / 2 \times t$$

Where

TS = tangential stress of the skin (MPa);

T = turgor pressure, CT_{50} (MPa);

r = radius of the fruit (cm);

t = thickness of the skin (cm)

Analysis

SPSS (version 15.0) was used for most statistical analyses. Regressions of climate data (for three weeks prior to harvest maturity) and cracking were calculated, and chi square was used to compare cracking frequency within seasons and varieties. Effects of aspect (north and south) and position (upper or lower branch) were assessed using pair-wise comparison.

Logistic regressions (SAS Version 9.1) were used to estimate CT_{50} for each variety over two seasons, and the predicted probabilities and log odds of crack types for each variety and season. All results quoted as ‘significant’ are at probability level of 0.05 or below.

Results

In 2004/05 there were three rainfall events during the critical preharvest period giving a total of 22 mm. The total rainfall from full bloom to harvest was 52 mm. Crack development and type was not significantly affected by position, data not shown. Cracks had developed in all varieties by the second sampling time, approximately two weeks prior to commercial harvest, (Figure 8). The proportion of fruit with cracks increased up to one week prior to commercial harvest, but in the days immediately prior to harvest, there was no further increase in cracking percentages except for the variety Summit (Figure 8). At relative maturity, all varieties showed similar development patterns. Apical and stem cracks were observed at the first sampling time, with side cracks taking several more days to develop and then increase from the third to final sampling time.

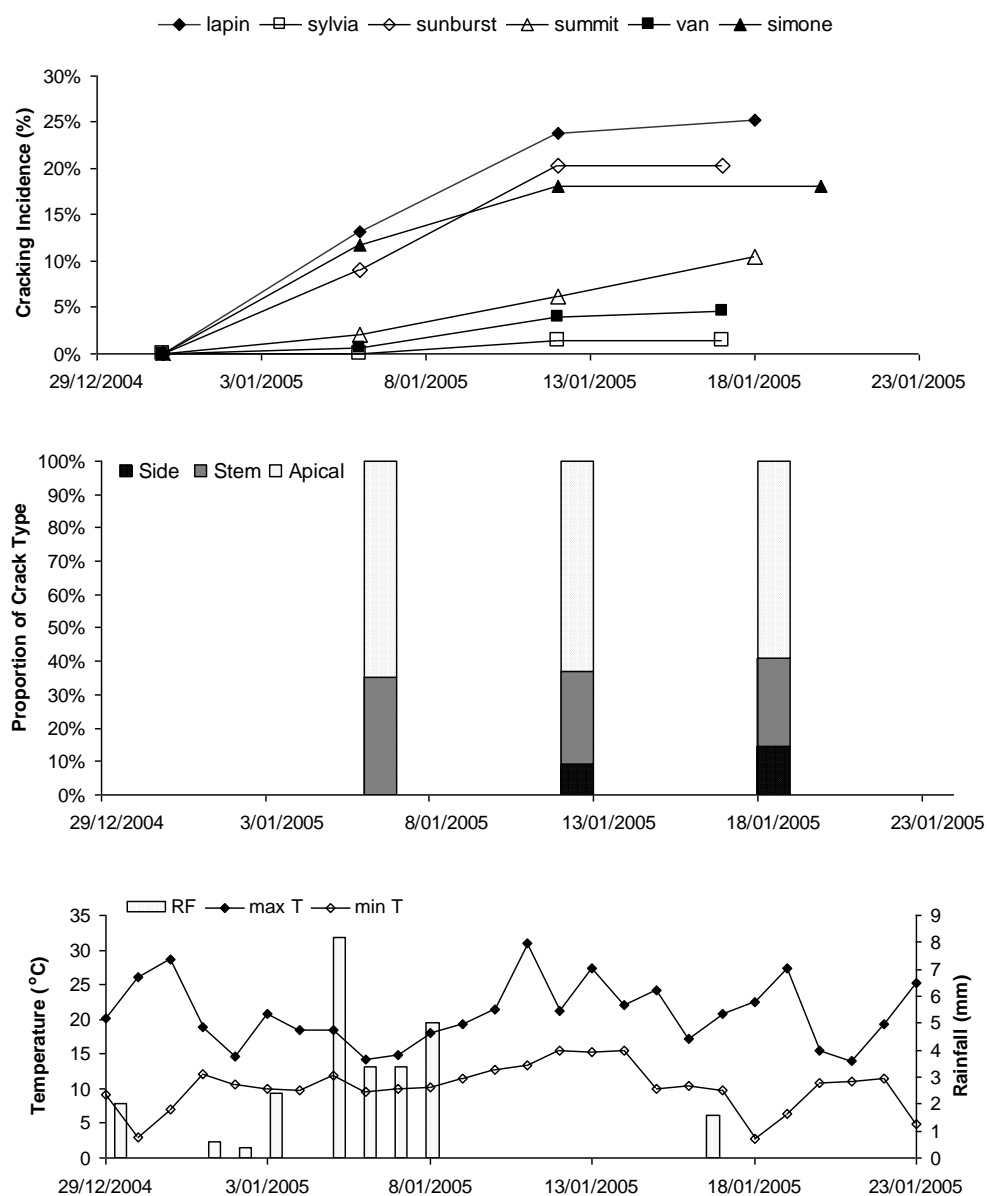


Figure 8 Cumulative cracking incidence and proportion of each crack type of six sweet cherry varieties over Stage III period of maturation, 2005. Daily maximum and minimum temperatures (°C) and rainfall (mm) over the same period.

Table 2 The percentage of fruit with cracks for each variety, over four seasons. The mean level of crack types for each variety and in each season is given. A significant relationship was found between both year and level of cracking, and between crack types within varieties. ($P < 0.05$).

Variety		2005	2006	2007	2008	Average
Lapins	Apical	20.7	11.4	3.5		11.9
	Stem	1.3	11.4	1.2		4.6
	Side	2.1	17.3	5.9		8.4
	Total	24.2	40.0	10.6		25.0
Sylvia	Apical	0.0	9.3	1.0	0.0	2.6
	Stem	0.0	8.3	0.5	0.0	2.2
	Side	1.4	21.8	3.5	0.0	6.7
	Total	1.4	39.4	5.0	0.0	11.5
Simone	Apical	16.6	10.3		0.1	9.0
	Stem	2.0	8.5		0.4	3.6
	Side	0.0	19.8		0.3	6.7
	Total	18.6	38.6		0.8	19.3
Summit	Apical	10.4		5.0	0.0	5.1
	Stem	0.0		0.1	0.0	0.0
	Side	0.0		0.0	0.0	0.0
	Total	10.4		5.1	0.0	5.2
Van	Apical	1.0		1.3	0.1	0.8
	Stem	2.2		0.0	2.1	1.4
	Side	1.4		2.1	1.4	1.6
	Total	4.6		3.4	3.7	3.9
Sunburst	Apical	0.4			0.0	0.2
	Stem	14.6			0.0	7.3
	Side	5.9			0.0	3.0
	Total	20.9			0.0	10.5
Kordia	Apical		4.1	0.0	0.0	1.4
	Stem		25.9	9.2	0.2	11.8
	Side		24.7	6.6	1.0	10.8
	Total		54.7	15.8	1.2	23.9
Regina	Apical		0.6	0.0	0.0	0.2
	Stem		0.9	0.1	0.2	0.4
	Side		21.6	13.0	4.4	13.0
	Total		23.0	13.1	4.5	13.5
Sweetheart	Apical		14.0	0.2	0.0	4.7
	Stem		7.0	6.0	0.0	4.3
	Side		15.7	4.9	0.0	6.9
	Total		36.7	11.2	0.0	16.0
Season Average	Apical	8.2	8.3	1.6	0.0	
	Stem	3.3	10.3	2.4	0.4	
	Side	1.8	20.1	5.1	0.9	
	Total	12.8^b	35.1^a	9.2^b	1.3^c	

A significant difference in total cracking levels between seasons was found (Table 2). However, there was no significant effect on the level of total cracking or individual crack types of either total rainfall or rainfall timing (number of rainfall days) during the critical period in all varieties. The lowest cracking level was in 2007/08 (1.3 %) and this season recorded the lowest rainfall in the three week period prior to harvest, with rainfall ranging from 3 to 14 mm depending on variety harvest date for all varieties except ‘Sunburst’ (45 mm), which was harvested eight days before the other varieties (06/01/2008) (Figure 9). The highest level of total cracking was recorded in 2005/06 (35.1 %), with lower levels recorded in 2004/05 and 2006/07 (12.8 %, and 9.2 % respectively). In 2004/05, 2005/06 and 2006/07 rainfall in the critical period was 7 to 22 mm, 6 to 36 mm and 26 to 67 mm respectively depending on variety harvest date (Figure 9).

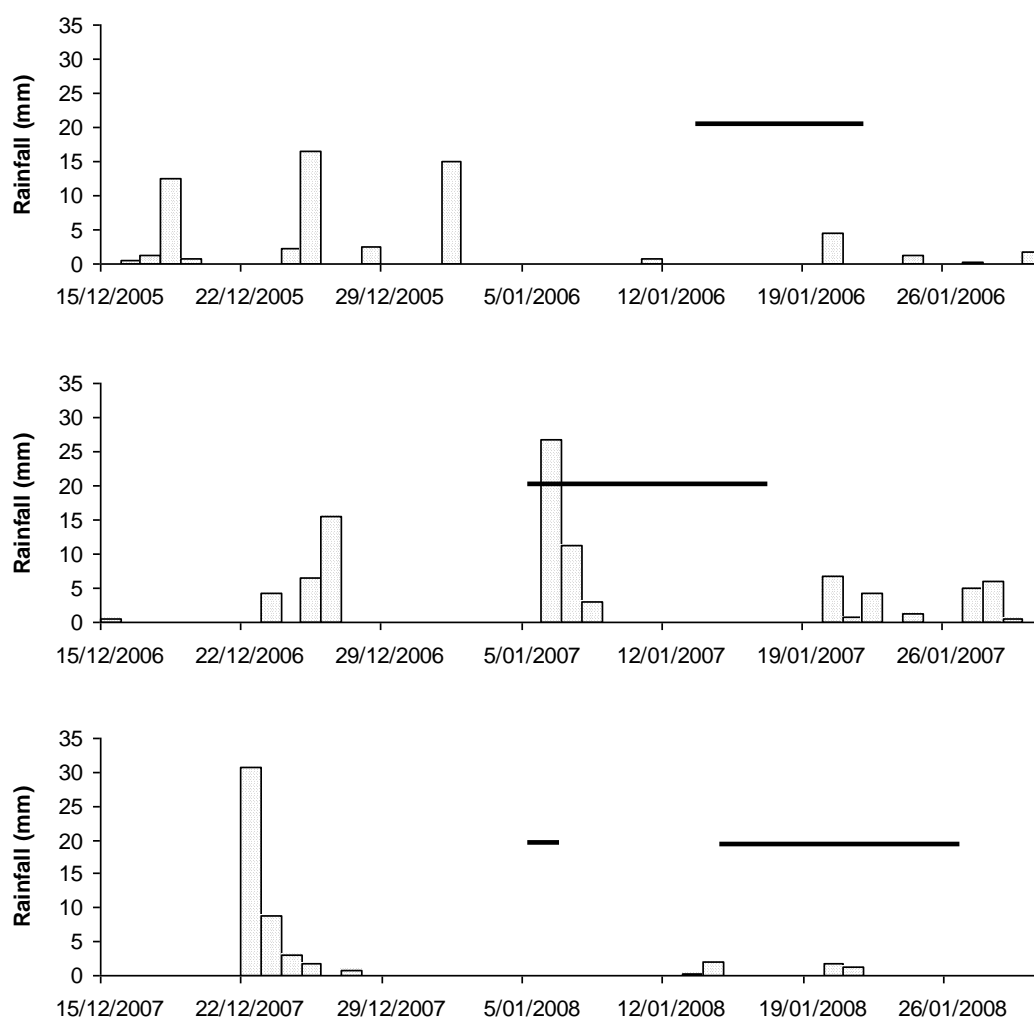


Figure 9 Rainfall during the critical preharvest period and harvest period for seasons 2005/06, 2006/07 and 2007/08. Harvest dates for each season are represented by the horizontal bar. Season 2007/08 had two distinct, separate harvest periods.

Considering data from all three seasons, there was a significant difference in the proportion of crack types found within all varieties, both within and between seasons. The Log odds (Figure 10) show season 2004/05 as the only season more likely to experience more apical cracks than either stem or side cracks.

A significant difference was found in the proportion of the various crack types between varieties and, with the exception of 'Van', between seasons within the same varieties. The predicted probabilities of crack type development (Figure 10)

show the variety ‘Summit’ (4) to have a high propensity for apical crack development, and more likely to develop apical cracks than stem or side cracks (Figure 10). ‘Sunburst’ (6) and ‘Regina’ (8) were most likely to develop stem and side cracks with few apical cracks.

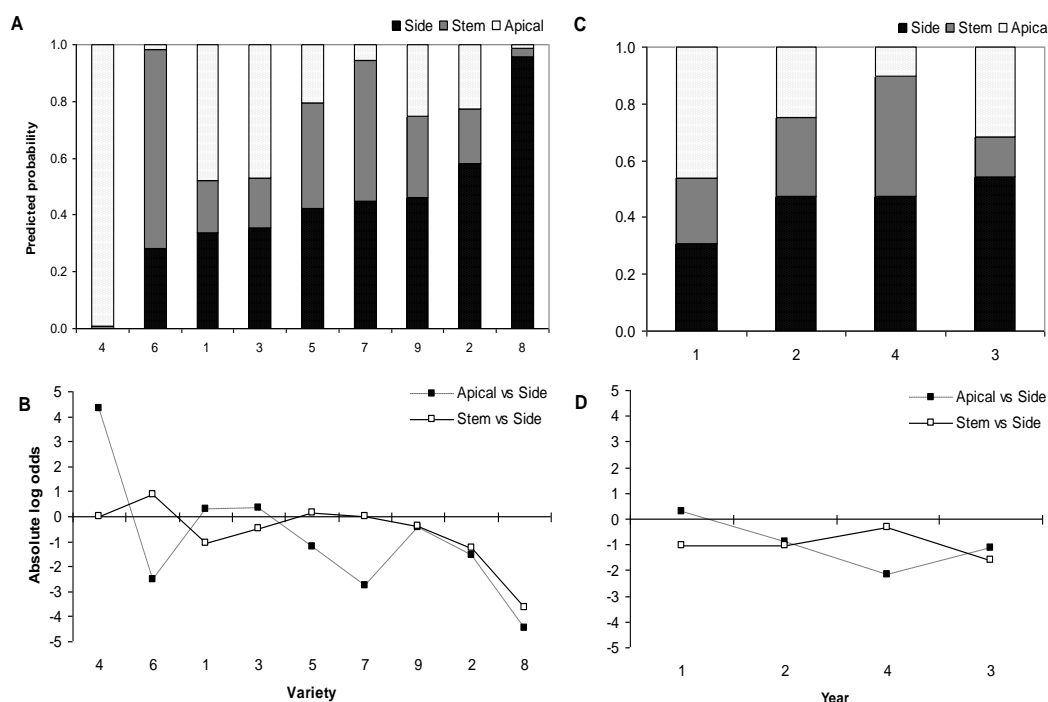


Figure 10 Predicted probabilities of crack types by variety (A) and season (C), and Log odds of cracks at stem or apical positions versus the side position by variety (B) and season (D). Data sorted in order of probability of side cracks. Varieties: 1-Lapins, 2-Sylvia, 3-Simone, 4-Summit, 5-Van, 6-Sunburst, 7-Kordia, 8-Regina, 9-Sweetheart. Seasons: 1-2004/05, 2-2005/06, 3-2006/07, 4-2007/08.

There were no relationships between fruit size, weight, Brix measurement or osmotic potential at harvest maturity and level of cracking within varieties (data not shown).

Critical turgor potentials for 50 % fruit cracking (CT_{50}) and Tangential Stress (TS) for seasons 2004/05 and 2006/07 are given in Table 3. A significant correlation

between actual field percentage of total cracked fruit at harvest maturity, and tangential stress at critical turgor was found ($R^2 = 0.56$) (Figure 11). A significant correlation between side cracks and tangential stress was found in 2006/07 only ($R^2 = 0.72$). No significant relationships were found with other crack types and tangential stress.

Table 3 Calculated turgor potentials (CT_{50}) with 95 % confidence limits (CL) and the tangential stress (TS) at which 50% of fruit cracked for each sweet cherry variety sampled at harvest maturity in production seasons 2004/05 and 2006/07.

2004/05	Variety	CT50	CL(95%)	TS (MPa)
	Lapins	3.78	± 0.1138	43.34
	Simone	2.23	± 0.3145	89.12
	Summit	3.16	± 0.5103	116.16
	Sunburst	2.92	± 0.3145	84.50
	Sylvia	2.75	± 0.1759	139.63
	Van	3.69	± 0.2205	165.17
2006/07	Variety	CT50	CL(95%)	TS (MPa)
	Kordia	2.89	± 0.1246	105.06
	Lapins	2.58	± 0.0418	89.48
	Regina	2.01	± 0.0181	75.93
	Summit	2.67	± 0.1085	110.91
	Sweetheart	2.32	± 0.1343	97.94
	Sylvia	2.42	± 0.1379	93.52
	Van	2.69	± 0.2352	107.69

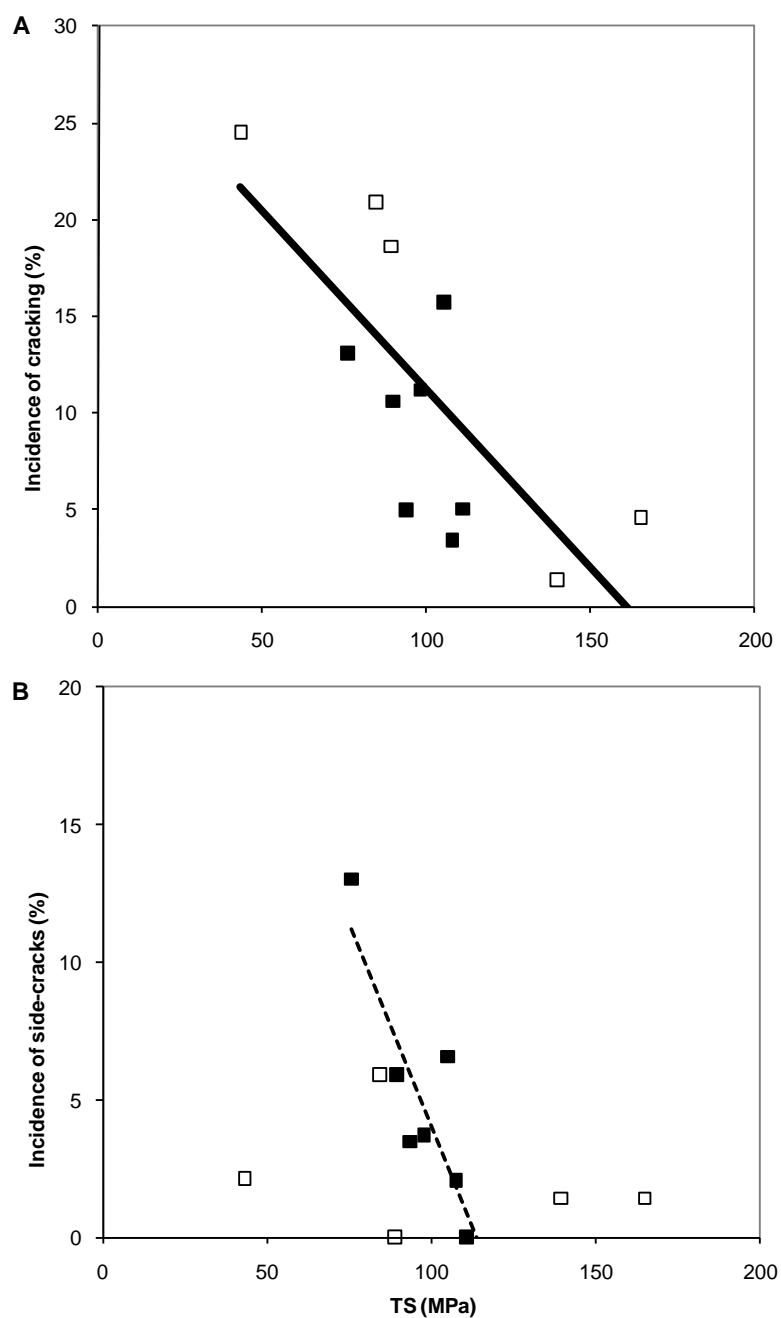


Figure 11 The relationship between the tangential stress (TS) experienced at CT50 and the percentage of fruit with (A) total cracks and (B) side cracks at harvest maturity for the varieties harvested in 2004/05 (□) and 2006/07 (■). A significant relationship between total cracks and tangential stress was found. A significant relationship between side cracks and tangential stress was found in 2006/07 only. Relationships are shown by solid line (total cracks) and dotted lines (side cracks 2006/07) ($P < 0.05$).

Discussion

Rain in the critical preharvest period (three weeks prior to commercial harvest maturity) was followed by cracking in all varieties, confirming the generally accepted link widely reported in the literature that cherry fruit cracking is rain-induced (Sekse 1995b, Lane *et al.* 2000). However, the extent of cracking (measured as total number of cracked fruit) did not show a link with the amount or timing of rain in this critical period. Although the lowest rainfall year (07/08) recorded the lowest cracking percentage, the worst cracking damage occurred in the year with second lowest rainfall in the critical period (05/06). These results suggest that while there is a clear link between rainfall and crack development, the response may not be a direct relationship. This supports the claim by Lane *et al.* (2000) of a strong environmental rather than anatomical influence on susceptibility.

Season (or year) strongly influenced both the proportion of different crack types and cracking levels. Data from 04/05 indicates that different crack types have different developmental patterns in response to rainfall. Apical and stem cracks were observed first, followed later by the larger side cracks. This suggests that proliferation of the smaller concentric stem and apical cracks and the deeper side cracks may be resulting from two independent processes.

There were similar trends in incidence of individual crack types within varieties, further indicating that each variety has a predisposition to a particular crack type. It can be concluded from these variety results that future cracking management

may need to become variety specific, and current cracking indices (Christensen 1996) may need to be revisited.

Differences in tensile strength of the fruit skin were a major determinant of total cracking incidence recorded in the field. The negative linear correlation between tangential stress and percentage cracking in the field (Figure 11) further indicates a varietal difference in overall propensity to cracking. These differences in propensity to total cracking incidence and crack type development are based on *in situ* conditions rather than laboratory immersion testing to determine cracking indices. Beyer *et al* (2002) (Beyer *et al.* 2002b) investigated regions of preferential uptake of water by cherry fruit, concluding that although these regions exist (pedicel ends), this finding may impact more on laboratory immersion testing where pedicels are removed, rather than actual field situations.

The significant relationship found between tangential stress and the incidence of side cracks in one year, and lack of the same relationship in the other year, further illustrates a seasonal and varietal effect that is not simply a relationship between rainfall and cracking. Sawada (1934) suggested that the location of cracks was determined by the shape and curvature of the fruit. The resultant stresses due to different shapes may explain the differences seen in this study. The investigation of a two dimensional tension test by Bargel *et al.* (2004) supports the need for an improved method of testing mechanical properties of plant materials such as cherry fruit skin in relation to cracking susceptibility. It has also been shown that cherry fruit cuticular conductance changes with periods of high growth rates (Knoche *et al.* 2001) and that fruit expansion induces strain of the cuticular membrane (Knoche *et al.* 2004). Microcracks are known to develop in the fruit

cuticle with increasing fruit maturity, increasing strain on excised segments of fruit skin (Peschel and Knoche 2005). Microcracks also show a varietal difference in development (Hovland and Sekse 2003). Investigation of the changes and development of tangential stress on skin of maturing fruit (on several varieties and over several different seasons) would be advantageous in increasing the knowledge of the underlying mechanical principles of fruit cracking resulting from water uptake across the fruit skin.

However, turgor pressure and osmotic potential differences between varieties were not related to total cracking or relative incidence of the three crack types, suggesting that large osmotic gradients across the fruit skin produced by high sugar levels may not be the driving force behind increased water entry. These findings concur with Sekse (1995a) who found that water entry may be via processes other than osmotically driven transcuticular flow. Different crack types in plums resulting from independent processes, was suggested in a study by Uriu *et al.* (cited in Christensen 1996).

The results of this investigation suggest that different crack types do not represent more or less severe expressions of the same processes, but may be symptoms of different water uptake pathways or mechanisms. A further study is proposed to assess the development of these different crack types in response to different water uptake pathways by the fruit, directly across the fruit surface and via the vascular system of the tree.

Furthermore, the seasonal and varietal influences on the incidence and type of crack, indicate that susceptibility to fruit cracking and the development of

individual crack types can no longer be determined by either fruit properties or environmental conditions alone, but must take an integrated whole tree approach to the problem. Continued work in this area would further the current understanding of the nature of fruit cracking in sweet cherries, particularly in the Australian climate, and may lead to variety specific management.

Conclusion

The development of different types of sweet cherry fruit cracking is the result of both variety traits and seasonal influence. The data demonstrate that varieties show a consistent pattern of individual crack type development, and season strongly influences both crack type and the total level of cracking observed. Future management of fruit cracking will need to consider varieties on an individual basis. The only fruit property to significantly explain total cracking levels between varieties was the tangential stress experienced by the fruit skin determined from a critical turgor at which 50 % of fruit cracked. Continued work in the field of fruit skin stress is warranted.

Chapter 5

Vascular Flow of Water Induces Side Cracking in Sweet Cherry (*Prunus avium* L.)

Given the distinct varietal propensity for cracking seen in the results presented in Chapter 4, and the lack of a significant relationship between cracking incidence and rainfall amount or distribution in the critical preharvest period, Chapter 5 reports the investigation into the development of different crack types, and the role (or otherwise) of an alternative excess water uptake pathway. This chapter addresses the following two experimental objectives;

- To investigate the development of different crack types
- To determine the importance of whole tree water relations (the alternative theory) to crack development

Introduction

Cracking in sweet cherries is a generic term used to describe rain-induced fracturing of the fruit skin, sometimes associated with rupturing of underlying flesh. Some studies have noted differences in cracking patterns (Sawada 1934, Christensen 1972) but most published work has not differentiated cracks in terms of size, shape, depth or location on the fruit surface. Sawada (1934) suggested that different forms of cracking might be related to fruit shape and Christensen (1972) noted varietal differences in cracking type and incidence. Chapter 4 examined cracking patterns more closely by comparing varieties across seasons and suggested that the crack types, previously defined by Christensen (1996), may be driven by separate mechanisms. This study proposes that these crack types fall into two broad but distinct categories: (1) large deep tissue failure often extending across the side of the fruit surface and (2) small shallow circular or semicircular cuticular fractures around the stem or apical ends of the fruit.

Many of the management practices (rain covers and spray applications) arising from previous cracking research have focused on preventing water uptake across the fruit surface and much of the published cherry cracking research (e.g. (Beyer *et al.* 2005)) has concentrated on skin permeability and other physical properties of fruit. Both management and research have concentrated on cracking resulting from excess water uptake directly across the fruit skin. Little attention has been paid to the whole tree hydraulic system, which was implicated as early as 1934 when swelling of cherry pulp through normal expansion was suggested as a cause of cracking (Kertesz and Nebel 1935). The possibility that water moving in the soil-plant-atmosphere system may be an alternative mechanism driving cracking

was raised by Sekse (1995a, b) but the suggestion has not yet been widely investigated. Uncertainty about pathways for water entry into plant tissue prone to structural failure has not been limited to sweet cherries, with papers on tomatoes (Ohta *et al.* 1998), capsicums (Aloni *et al.* 1999) and carrots (Gracie and Brown 2004) reporting or implicating the plant hydraulic system. To further develop effective management strategies for cracking, the relationship between crack prone fruit and environment (including factors influencing water uptake and flow) requires further investigation.

The present study was designed to establish how water uptake by fruit, either internally through the vascular system, or externally across the fruit surface, affects the incidence and types of cracks formed. Given the findings presented in Chapter 4, that varieties differ in both overall propensity to cracking and the ratio of crack types developed, the results of this study may have implications for variety specific rain-induced cracking management.

Materials and Methods

Plant Material

Five trials were undertaken. Three trials were designed to separate the cracking effects resulting from water application to different water uptake zones (i.e. the root-zone and the canopy). During the first of these trials, using trees from the surrounding orchard, sap flow in fruit pedicels and adjacent leaf petioles was measured to obtain a picture of diurnal sap flow in response to rainfall. Another trial investigated the effects of time of water application on cracking incidence and type. A smaller trial examined the role of spur leaves in crack development.

Mature trees on F12/1 rootstock pruned to a Spanish bush system, with intra-row spacing of approximately 2 m, were used in all trials. Cultivar „Simone’ was used for the first three water application trials and cultivar „Lapins’ was used in the other two trials. Both varieties are susceptible to all types of cracking (Chapter 4). Trials were located in a commercial orchard and a neighbouring research orchard near Huonville in southern Tasmania (Australia) (42°98'S, 177°08'E). Soils on the two sites were both brown sodosols typical of the area (Wilson *et al.* 2004) and both orchards were subjected to standard industry fertiliser, dripper irrigation and pest and disease control regimes.

Water Uptake Zone Trial

To examine the separate and combined effects of water applied to the ground and to the canopy, simulated rain trials were conducted in 2005/06 (Trial 1), 2006/07

(Trial 2) and 2007/08*¹ (Trial 3). In each trial, treatments were applied using micro sprinklers situated under or over the canopy and, where required, clear plastic mulch was used to prevent canopy applied water entering the root-zone of the trees. To simulate a single rainfall event, water (equivalent to 30 mm rainfall) was applied over approximately five hours during the morning on a fine, cloudless day, between four and seven days before anticipated harvest maturity. Timing of water application was designed to allow for a rain free period of four days prior to application until harvest. Water applied in each trial was additional to the normal commercial irrigation schedule. Harvest occurred four days post application. All fruit from all trees was harvested, counted and separated into side, stem or apical crack types, and fruit weight and diameter determined as described in Chapter 3. Fruit was then frozen for later measurements of osmotic potential using the methods described in Chapter 4.

Treatment design in each trial was a 2x2 factorial as follows; water applied or not applied to the canopy and water applied or not applied to the ground. Prior to treatment, trees were blocked into groups based on trunk girth. There was a minimum buffer of two untreated trees between treatments. Six replicates were used in seasons 2005/06 and 2007/08 and seven in 2006/07, all in randomised complete block designs.

¹ The trial was also undertaken in Campania (Site 3), but was aborted due to heavy rainfall.

Sap Flow Monitoring

Sap flow direction and magnitude in fruit pedicels and the adjacent leaf petioles were monitored. A total of four full 24 hour days were monitored for net sap flow patterns, with results covering days without rain and one natural rain day.

Sap flow meters IVP-4, obtained from the “Biopribor” Design Bureau of the Moldavian Academy of Sciences, Kishinev, were used (Shabala 1997). Prior to installation gauges were calibrated at controlled pressures using fresh cherry fruit pedicels excised under water. In use, gauges were covered in aluminium foil as a solar radiation shield and were positioned to monitor sap flow through three fruit pedicels, and three leaf petioles per tree. An additional sensor was placed in the tree canopy, but not attached to pedicel or petiole, to measure any extraneous effects of light, wind or rain. Data were recorded every 5 minutes and converted to flow rate (ml/hr).

Water Application Timing Trial

A fourth water application (simulated rain) trial was conducted in 2007/08 (Trial 4). A single simulated rain treatment, with water applied to both canopy and ground, as in previous trials, was applied at different times throughout a 24 hour period. Treatment times (AEDT – Australian Eastern Daylight Time) were morning (7 am -12 pm), afternoon (2 pm -7 pm) and night (12 am – 5 am), giving three rainfall treatments and an untreated (no rain) control. Cracking and fruit properties were subsequently assessed as for Trials 1, 2 & 3. There were six

replicates in a randomised complete block design, with blocking based on trunk girth. Treated trees were buffered as for Trials 1, 2 & 3.

Spur Leaf Manipulation and Fruit Cracking

To investigate the role of spur leaves on fruit cracking, a trial (Trial 5) undertaken in 2004/05 compared removal of spur leaves (LR), retention of spur leaves which were tied back to expose fruit (LP) and an untreated control. Treatment LR consisted of excising all spur leaves within 20 cm on either side of a fruit cluster and treatment LP consisted of carefully tying the leaves back from around the cluster using string. Both treatments exposed the fruit directly to natural rainfall for the duration of the trial. Treatments were applied three weeks prior to harvest and fruit cracking monitored weekly. Cracked fruit were tagged, and cracks classified into apical, stem or side cracks and counted. All fruit were left on the tree until harvested at commercial maturity. Fruit were then frozen for later measurements of osmotic potential using the methods described above.

Trial design was a randomised complete block, using six trees as blocks with the three treatments allocated at random to single branch plots.

Rainfall figures for all trials were obtained from the Australian Bureau of Meteorology Station at Grove, less than 1 km from both trial orchards.

Statistical Analysis

A square root transformation was applied to data for analyses to meet the assumptions of the ANOVA and linear regression tests. Transformed data were

subject to ANCOVA (crop load as the covariate) in the water application trials using PROC GLM. Crop load was defined as the number of fruit per cross-sectional trunk area. PROC GLM was also used in the analysis of the leaf manipulation trial and the analysis of fruit osmotic potential. Treatment means were compared using Fisher's protected LSD. Statistical software SPSS (version 17) and SAS (version 9.1) were used in this study. Unless specified, all results quoted as „significant' are at probability level of 0.05.

Results

Canopy and Root-Zone Water Application Trial

While the incidence of total fruit cracking in the field varied season to season (2005/06 > 2006/07 > 2007/08), similar patterns of crack type development in response to water application treatments were evident. There was a significant ($P < 0.01$) main effect of root-zone applied water on the incidence of side crack development in all years (Figure 12). There was also a significant main effect of canopy applied water on cuticular crack development, for apical cracks in all years and on stem cracks in seasons 2005/06 and 2007/08 (Figure 13). A significant ($P = 0.04$) interaction between canopy and root-zone applied water in the development of stem-end cracks occurred only in 2006/07, with an increased, but not significant, incidence in this crack type evident in all treatments. The osmotic potential of side-cracked fruit was found to be significantly lower than fruit with either cuticular cracks or no cracks (Table 4).

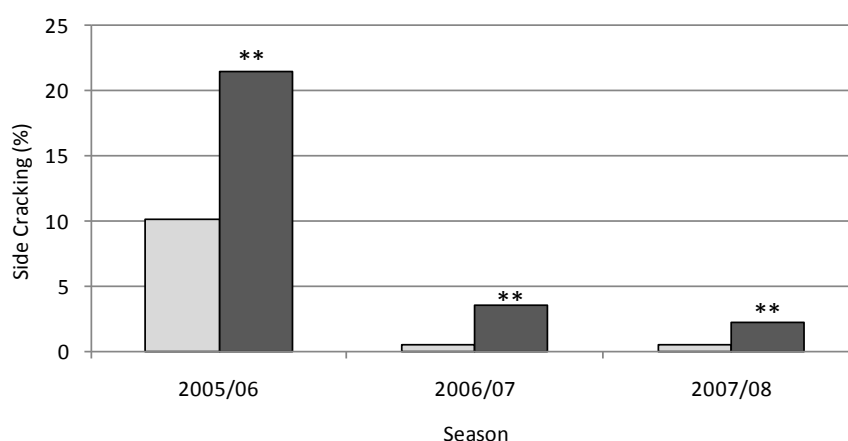


Figure 12 Mean percentage of fruit with side cracks when water was applied (dark column) or not applied (light column) to the tree root zone. ** indicates a significant difference at the 0.01 level between the two treatments.

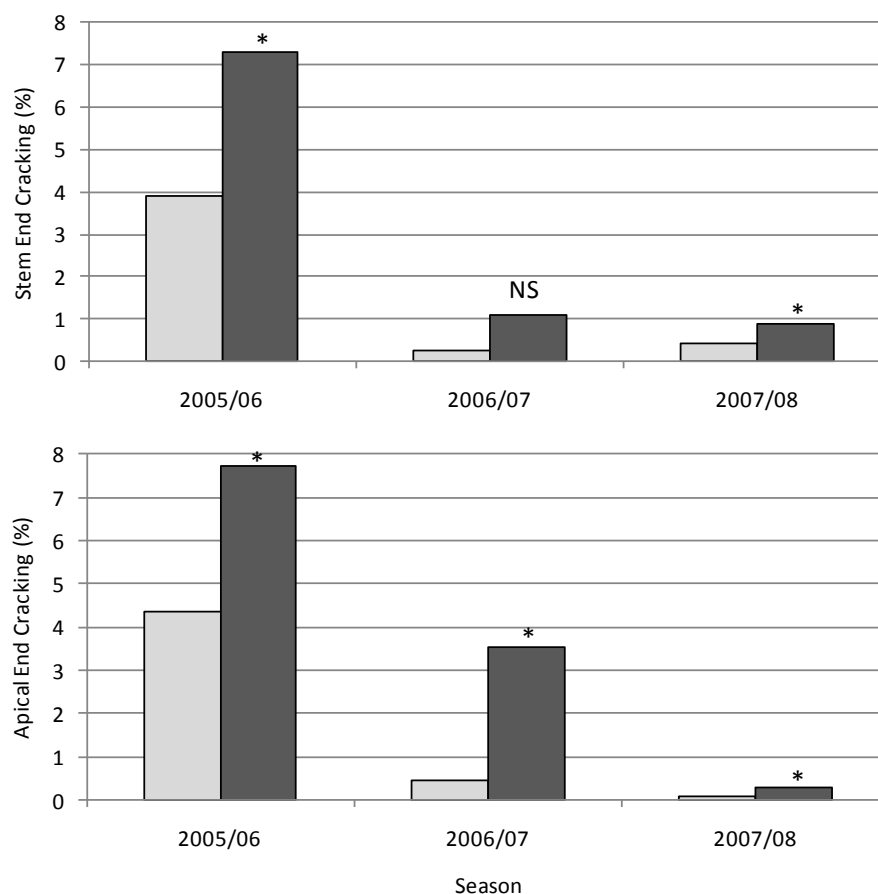


Figure 13 Mean percentage of fruit with stem-end and apical-end cracks after water was applied (dark column) or not applied (light column) to the tree canopy. * indicates a significant difference at the 0.05 level between the two treatments.

Table 4 Mean osmotic potential values for fruit of different crack types and non-cracked fruit with 95% Confidence Intervals (CI) from (A) the water application trials and (B) the untreated control from the leaf manipulation trial. A total of 358 and 48 fruit were measured respectively.

Crack Type	Osmotic Potential (MPa)	CI (95%)	Osmotic Potential (MPa)	CI (95%)
	A.		B.	
Non Cracked	-3.33	±0.12	-3.36	±0.18
Stem End	-3.36	±0.13	-3.34	±0.27
Apical End	-3.32	±0.10	-3.29	±0.13
Side	-3.71	±0.15	-4.39	±0.47

Sap Flow Monitoring

Only one rain event (16 mm) was recorded during the critical preharvest period, allowing for monitoring of flow in a natural rainfall event on one day only. Figure 14 shows daily patterns of net sap flow for fruit and for leaves on the same spur from a typical dry day, and from a day experiencing natural rainfall of 16 mm that commenced at 3 pm (AEDT). Compared with rain free days, natural rainfall showed an influence on directional flow with consistently increased influx to the fruit during daylight hours. There was a sharp influx to the fruit after commencement of afternoon rainfall (Figure 14). The rate of sap influx was low during the night (10 pm to 6 am) in both the fruit pedicel and leaf petiole, irrespective of rainfall.

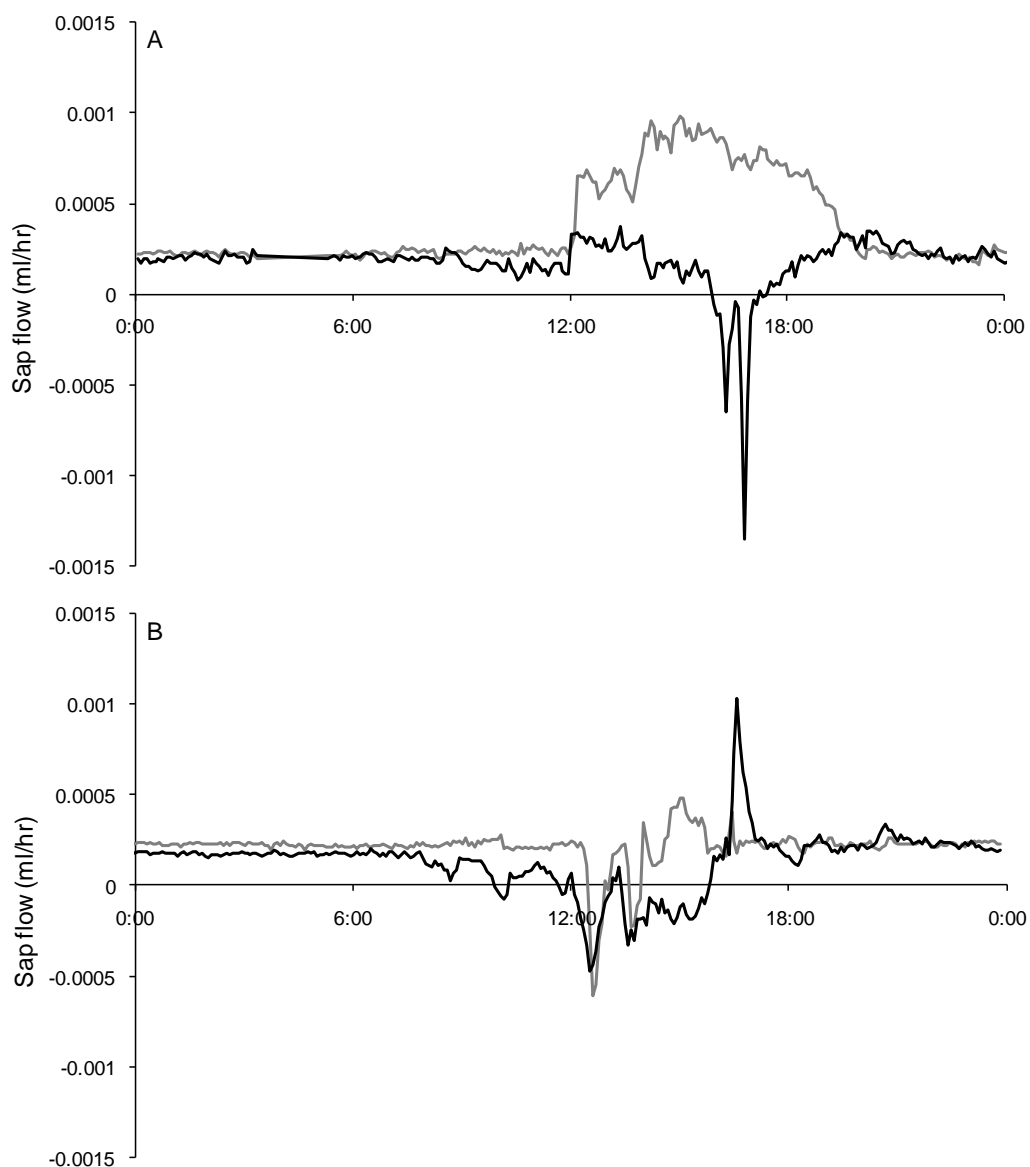


Figure 14 Net sap flow rate through leaf petioles (pale lines) and adjacent fruit pedicels (dark lines) on a single tree; (A) on a typical day without rain (24 hours), and (B) on a day (24 hours) with 16 mm rain commencing mid afternoon. Positive flow rate indicates a net movement of water into the fruit/leaf (influx), while a negative flow rate indicates a net movement out of the fruit/leaf (efflux).

Water Application Timing Trial

Although the overall level of cracking in Trial 4 was low, there was a significant ($P < 0.01$) effect of treatment within the trial day, on the development of side cracks (Table 5). Highest levels of side cracking were produced by afternoon simulated rainfall applications and there was a significant treatment effect on the development of stem-end cuticular cracks but not apical-end cuticular cracks. There was no significant difference in cracking incidence between the night treatment and the control (Table 5).

Table 5 Percentage of fruit (untransformed data) with either apical, stem or side cracks, resulting from different time of water application treatments. Within columns, treatments with the same letters are not significantly different ($p < 0.05$) from each other based on analyses performed on transformed data ($n=6$).

Time of Application	Crack Type			Total
	Apical	Stem	Side	
No water	0.0	0.5 ^a	0.5 ^a	1.0 ^a
Night	0.0	0.5 ^a	1.2 ^{ab}	1.7 ^{ab}
Morning	0.1	0.8 ^a	1.9 ^{bc}	2.8 ^{bc}
Afternoon	0.0	1.7 ^b	2.8 ^c	4.5 ^c

Spur Leaf Investigation

During this trial, there was a total of 25 mm of natural rainfall, falling on eight separate days (Figure 3). The level of side-cracked fruit was significantly lower in both the LR treatment (0%) and the LP treatment (1.5%) compared with the untreated control (14%) (Figure 15). In contrast, a significantly ($P < 0.01$) higher proportion of fruit with apical-end cuticular cracks occurred in both the LR (79%) and LP (47%) treatments compared with the untreated control (32%) (Figure 15).

No significant difference in the proportion of stem-end cracks was recorded between the two treatments and control.

There was no significant ($P=0.34$) treatment effect on the osmotic potential of fruit. Due to low levels of side-cracked fruit in the two leaf manipulation treatments, only fruit from the untreated control was analysed for osmotic potential differences associated with crack type. The osmotic potential of side-cracked fruit was found to be significantly lower than for fruit with either cuticular cracks or no cracks (Table 4).

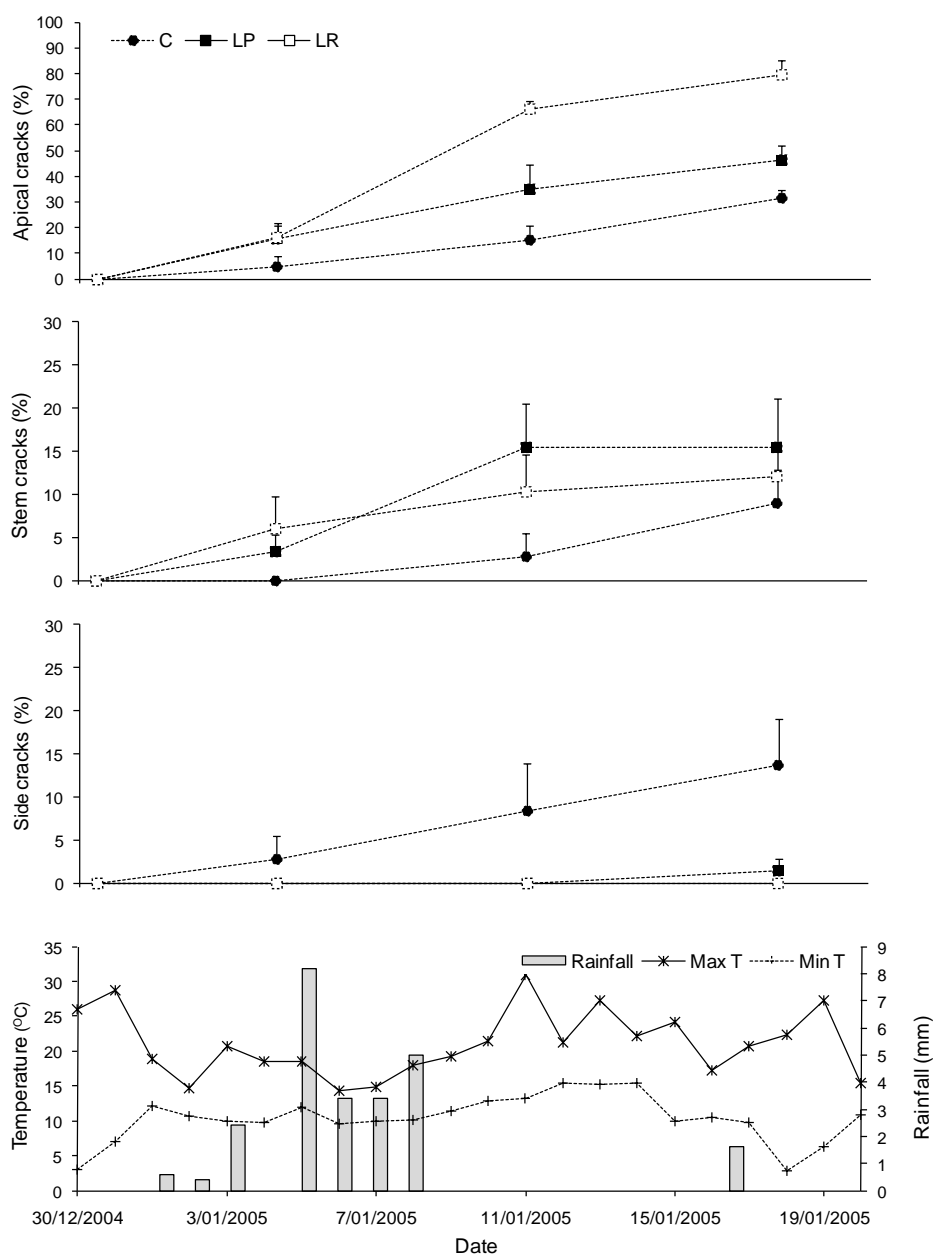


Figure 15 Cumulative percentage of fruit crack types with leaf treatments LR and LP and untreated control (C). Each point represents the mean percentage of fruit with cracks, error bars represent the SEM for each data point (n = 6).

Discussion

Equivalent but separate applications of water to canopy or roots in trials undertaken over three years confirmed that large, deep cracks on the cheek of the fruit are clearly associated with water applied to the tree root-zone, whereas cuticular cracks at the apical and stem-end of fruit were induced when water was applied to the tree canopy. The notion that different mechanisms underpin side crack and cuticular crack development is supported by the results of all trials in this study.

The increase of side cracks in response to root-zone applied water indicates that, in this situation, any excess water that enters the fruit to drive cracking must do so via the vascular system. This posit is further supported by the sap flow patterns in the fruit pedicel recorded in this study, which showed a high net influx of water to the fruit following natural rainfall. The actual pathway of this excess water is not yet fully explained.

After rainfall, influx via the phloem could be the pathway for the excess water that results in side-cracked fruit in this study. Fruit transpiration has been suggested as a regulator of diurnal water movement by initiating flow into tomato fruit through the phloem (Lee 1989). This is less likely to have a regulatory influence in cherries as tomatoes have considerably greater stomatal density (Gay and Hurd 1975) than cherries (Glenn and Poovaiah 1989). In this study, side-cracked fruit only occurred in conditions allowing normal leaf function, thus osmotic potential may influence the gradient by favouring flow to the fruit after rainfall. However with leaf removal, no significant increase in fruit osmotic

potential was found, supporting the findings of Ayala and Lang (2008) that both spur and shoot leaves and current season shoots are significant contributors of assimilates. Ayala and Lang (2008) showed that fruit are a priority sink within cherry trees, with the highest sink strength occurring during Stage III (maturation) of fruit development. In all trials, a significantly lower osmotic potential was recorded in fruit with side cracks compared with both cuticular-cracked and non-cracked fruit, however it remains unclear whether the lowered osmotic potential recorded developed prior to or following crack development.

On days without rain, the diurnal net sap flow patterns of the fruit pedicel and the adjacent leaf petioles are consistent with potential gradients essentially controlled by leaf evaporative demand. A close link between fruit and leaf water flux across potential gradients is supported by the work of Ohta *et al.* (1998) who noted that increased evaporative demand attributed to light induced leaf transpiration resulted in water efflux from tomato fruit. Turgor regulation in cherries could also be limited due to fruit pedicel xylem losing functionality as maturity approaches (Kozlowski 1968). Choat *et al.* (2009) suggest that while xylem hydraulic resistance increases with maturity, this did not fully explain the decline in xylem influx, concluding that berries are „hydraulically buffered’ by water flow in the phloem. This supports the claim by Lee (1989) that demand for water in tomato fruit links xylem water influx and phloem dry matter influx.

Results of this study suggest that side cracks are driven by vascular induced tissue expansion and are influenced by ambient diurnal conditions at the time of rainfall, perhaps explaining why fruit cracking in the absence of surface water has been

demonstrated for cherries under covers (Cline *et al.* 1995a), for capsicum (Aloni *et al.* 1999) and for tomato (Ohta *et al.* 1998).

Results also suggest that cuticular cracks are less likely to be driven by vascular water movement, but support the long held theory that fruit crack as a result of water movement across the fruit surface. Water application to the tree canopy resulted in cuticular cracks at both the apical end and the stem end of the fruit, however no difference in osmotic potential was recorded between cuticular cracked and non-cracked fruit.

Thus, rather than a vascular interaction with the adjacent spur leaves or osmotic driving of water across the fruit surface, the stronger influence in cuticular crack development may be surface wetting. Beyer *et al.* (2005) suggested cuticular cracking may occur when osmotic uptake in wetted areas is greater than transpiration in non wetted areas. Altered cuticular properties due to duration of fruit surface wetting, or increased levels of light and temperature may also influence cuticular uptake. Yao *et al.* (2000) and Moreshet (1999) both noted that shaded fruit (capsicums, apples, tomatoes, cherries) experienced less cracking than exposed fruit, due to lower temperatures and decreased exposure to direct irradiance thus maintaining cuticular structure. Exposed bell pepper fruit were found to have a more inelastic cuticle (Yao *et al.* 2000). In this study, fruit exposure resulted in an increased incidence of apical-end cracks, while afternoon water application resulted in an increased incidence of stem-end cracks.

The rate of water uptake across the fruit skin has also been suggested as having a greater effect on the likelihood of cracking than the actual amount of water (Cline

et al. 1995b). However, this could equally apply to the rate of water uptake through the internal vascular system, as suggested by Sekse (1995b) (Sekse 1995a). This is indicated in this study through the observed peaks in influx to the fruit following rainfall. Furthermore, it supports Sekse *et al.* (2005) who suggested uptake across the fruit surface contributed to an increased internal turgor pressure but that the dominant contributor to turgor is sap import. The research presented here adds further to this by clarifying pathways and crack types.

The two modes of water uptake resulting in different crack types may explain the inconsistency of results from cracking research to date. For example, studies that support a link between water and osmotic concentration of the fruit (Sawada 1934, Cline *et al.* 1995b) may be recording side cracks, while the findings of other studies that do not support this link (Kertesz and Nebel 1935, Christensen 1972, Moing *et al.* 2004) may be recording mainly cuticular cracks.

Given the propensity of different varieties to each crack type and that side cracks can make up the majority of total cracks in some varieties (Chapter 4), confirmation of a second source of water uptake in relation to cracking indicates that future management of cracking needs to be variety specific by targeting the appropriate mode of water entry.

Chapter 6

Diurnal Drivers of Vascular Flow in Sweet Cherry (*Prunus avium* L.).

This chapter explores the possible vascular pathways responsible for excess flow of water to the cherry fruit that results in cracking. This investigation is warranted given that the results of Chapter 5 implicate vascular flow as the mode of water entry inducing side-crack development in fruit following rainfall. The present chapter addresses the following experimental objective;

- To elucidate the diurnal fluctuations in fruit and leaf water relations

Introduction

Research into rain-induced sweet cherry fruit cracking has had a long history of investigating fruit and skin properties. The results of Chapter 4 have shown that the cracking incidence and the development of different crack types is influenced by both genotype and season, and Chapter 5 showed that large longitudinal „side’ cracks result from water supplied to the root-zone, entering fruit via the tree vascular system. At this stage, it is unclear what provides the driving force behind such flow patterns. Given the spikes in vascular influx observed following rainfall, and the differences in crack development with leaf removal or coverage, it is hypothesised that increased flux to the fruit could result from diurnal fluctuations in fruit and leaf water potential.

It is widely accepted that tension caused by potential gradients between the soil, through the plant, and to the ambient environment is the major driving force for water movement through plants, in line with the Cohesion Tension theory of sap ascension (Zimmerman *et al.* 2002). Further to this, fruit are natural reservoirs of water, which can be drawn upon when demand in the transpiration stream is high. Thus, flow to the fruit is influenced by multiple factors such as changing diurnal water potentials between the fruit and the leaf (Morandi *et al.* 2007), changing diurnal light intensity (Yamasaki 2003) and by source – sink interactions (Zhang *et al.* 2006). Cherry fruit are strong sinks (Ayala and Lang 2008) and therefore could influence magnitude of diurnal flow patterns.

Diurnal patterns of water potential have been recorded in other fruits, such as peach (McFayden *et al.* 1996), apricot (Alarcón *et al.* 2003) and grape (Greenspan

et al. 1994), as affecting diurnal changes in volume and diameter of growing fruit such as shrinkage during daylight hours followed by expansion. More severe cracking occurs in bell pepper fruits that had experienced higher diurnal amplitudes of expansion and shrinkage (Yao *et al.* 2000). Fruit diameter variation has been explained by Morandi *et al.* (2007) as the contribution of phloem import, xylem import and export, and transpiration export of water and it has been suggested by the authors that phloem and xylem are driven by both osmotic and turgor gradients. The relative contribution however, of the phloem and xylem to the fruit cracking process is unknown.

Given that the internal excess supply of water to the fruit during the afternoon resulted in increased cherry fruit cracking as observed in Chapter 5, the driving force behind this movement must be determined. This study aims to further investigate the diurnal factors influencing potential gradients and water movement into sweet cherry fruit, particularly in response to environmental parameters.

Materials and Methods

Plant Material

Mature trees, variety „Simone’, grown on F12/1 rootstock in a commercial orchard in Tasmania (Australia) (42°98'S, 177°08'E) subjected to standard industry management practices were used in this study. Diurnal fluctuations in leaf and fruit water potential and sap flow were investigated from late November to late January during 2005/06, 2006/07 and 2007/08. The influence of afternoon rainfall on leaf and fruit water status was also investigated in a simulated rainfall trial.

Diurnal Fluctuations in Water Potential and Sap Flow

On selected days in seasons 2005/06, 2006/07 and 2007/08 fruit (Ψ_F) and leaf (Ψ_L) water potentials were measured at regular intervals over several 24 hour periods giving a total of twelve full periods over the three seasons (four days, with at least one day with rainfall occurring, per season). To gauge the response of water movement to rainfall, sap flow direction and magnitude in fruit pedicels and the adjacent leaf petioles were also monitored in all three seasons (2005/06, 2006/07, and 2007/08) giving a total of thirty-seven full 24 hour periods covering days both with and without rainfall. In season 2007/08 spur xylem potential (Ψ_S) was also measured. This enabled the water potential gradients between fruit and leaf xylem ($\Psi_F - \Psi_L$), fruit and spur xylem ($\Psi_F - \Psi_S$), and between leaf and spur xylem ($\Psi_L - \Psi_S$) to be determined. Sap flow was compared with potential gradients on three days during 2007/08. These days (5th, 14th and 19th Jan or Day

1, Day 2 and Day 3) were chosen to represent different climatic conditions; Day 1 was hot, dry and sunny, Day 2 was mild, sunny with some cloud cover, and Day 3 was cool, overcast, and experienced a small (1.2mm) of rain.

Influence of Afternoon Rainfall on Diurnal Fruit Water Status

In 2007/08, simulated afternoon rainfall was applied to selected trees on one day in a randomised complete block design, with six whole tree replicates. Treatments consisted of simulated rainfall (approximately equivalent to 30 mm) commencing at 1 pm applied with micro irrigation (as per Chapter 5), and an untreated control. Fruit (Ψ_F), leaf (Ψ_L) and spur xylem (Ψ_S) water potentials were measured at regular intervals. The fruit samples used for water potential measurements on this day were also used to determine osmotic (π) and turgor (T) potentials.

Measurements of Water Relations

Water potential measurements were made in the field using a portable pressure chamber Instrument (Model 615, PMS Instruments). On each sample day five samples were randomly selected from fruiting spurs at each time interval starting pre-dawn. Fruit and leaf potential was measured from individual fruit and leaves respectively, immediately following excision from the tree using a sharp scalpel. Spur xylem potential was measured using leaves which had been covered with foil and sealed in plastic bags the previous evening. Sap flow monitoring was undertaken using equipment and method as described in Chapter 5 (page 89).

Osmotic potential of fruit was determined using a Wescor 5520 VAPRO® Vapor Pressure Osmometer as described in Chapter 4 (page 68) The turgor pressure (T) experienced by each fruit from each sample time was then calculated as:

$$T = \psi_f - \pi$$

Climatic Variables

Vapour pressure deficit (VPD) was calculated for trial days, from climate data (temperature and humidity) obtained from the Australian Bureau of Meteorology Station at Grove, less than 1 kilometre from the trial orchard. VPD was determined using;

$$VPD = e_{\text{sat}} - e_{\text{air}}$$

where e_{sat} is the saturation vapour pressure and e_{air} the air vapour pressure determined as per (Murray 1967).

Statistical Analyses

Statistical software package SPSS (version 15.0) was used to assess the effect of natural rainfall on the difference in water potential between fruit and leaves; ANOVA was performed with means compared using Fisher's protected LSD. Frequency analysis in SAS (version 9.1) was used to compare directional flow in fruit for days (8am -8pm) with and without rainfall (daytime values were used as night flows remained relatively constant). The Cochran-Mantel-Haenszel (CMH) test was used to determine the association between rainfall and direction of sap

flow, after controlling for the effect of year. Unless specified, all results quoted as 'significant' are at probability level of 0.05.

Results

Diurnal Fluctuations in Water Potential and Sap Flow

In all seasons, the most negative fruit water potential occurred in mid afternoon when the magnitude of fruit water potential (Ψ_F) was greater than leaf water potential (Ψ_L). On dry days the mean absolute difference between fruit and leaf potential ($\Psi_F - \Psi_L$) during the mid afternoon period was 0.55 MPa (Figure 16). However, on wet days, this difference was diminished to almost 0 MPa. Analysis showed that there was a significant ($P < 0.001$) difference in this potential gradient between wet and dry days (Figure 16). The diurnal fluctuations of total water potential patterns for leaves and fruit followed typical patterns; leaf and fruit water potential became increasingly negative during the hotter middle parts of the day, with recovery occurring during the afternoon and evening (Figure 17A, Figure 18A, Figure 19A).

Frequency analysis (CMH) of all days monitored showed a significant ($P < 0.0001$, $n = 3478$) association between the incidence of natural or simulated rainfall and the direction of sap flow to the fruit and leaves during daylight hours (8 am - 8 pm), after adjusting for year. On days with rainfall, sap influx was 7.98 ± 1.33 times more likely to occur than efflux compared to days without rainfall, where influx was only 0.32 ± 0.02 times more likely to occur than efflux.

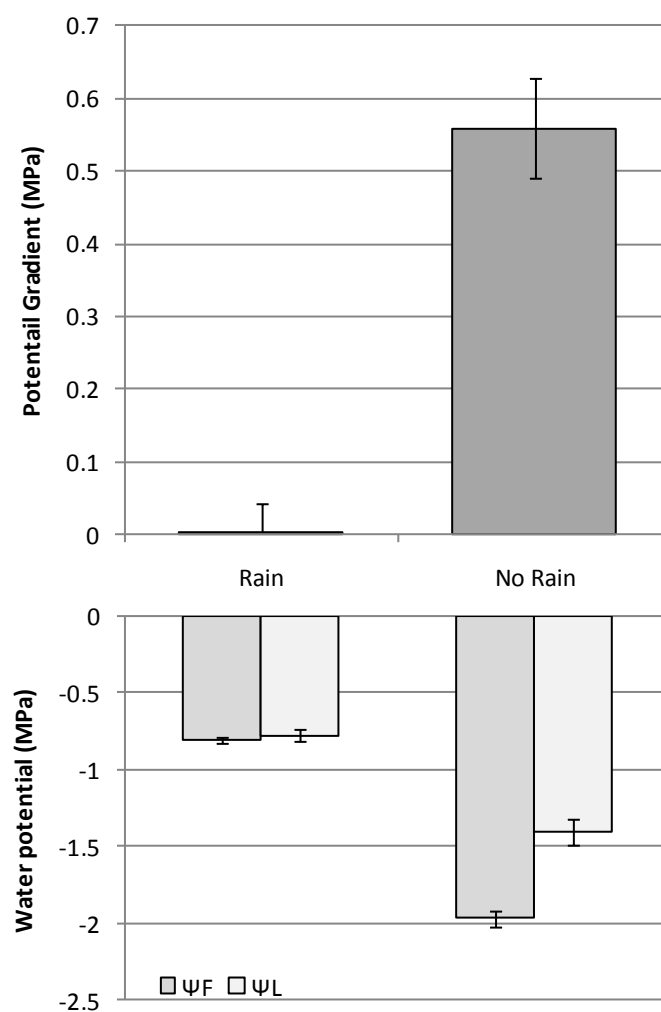


Figure 16 Mean absolute potential gradient between fruit and leaves ($\Psi_F - \Psi_L$) with mean fruit (Ψ_F) and leaf (Ψ_L) water potentials on days with rainfall ($n = 4$) and days with no rain ($n = 8$). Error bars indicate the standard error of the mean (SEM).

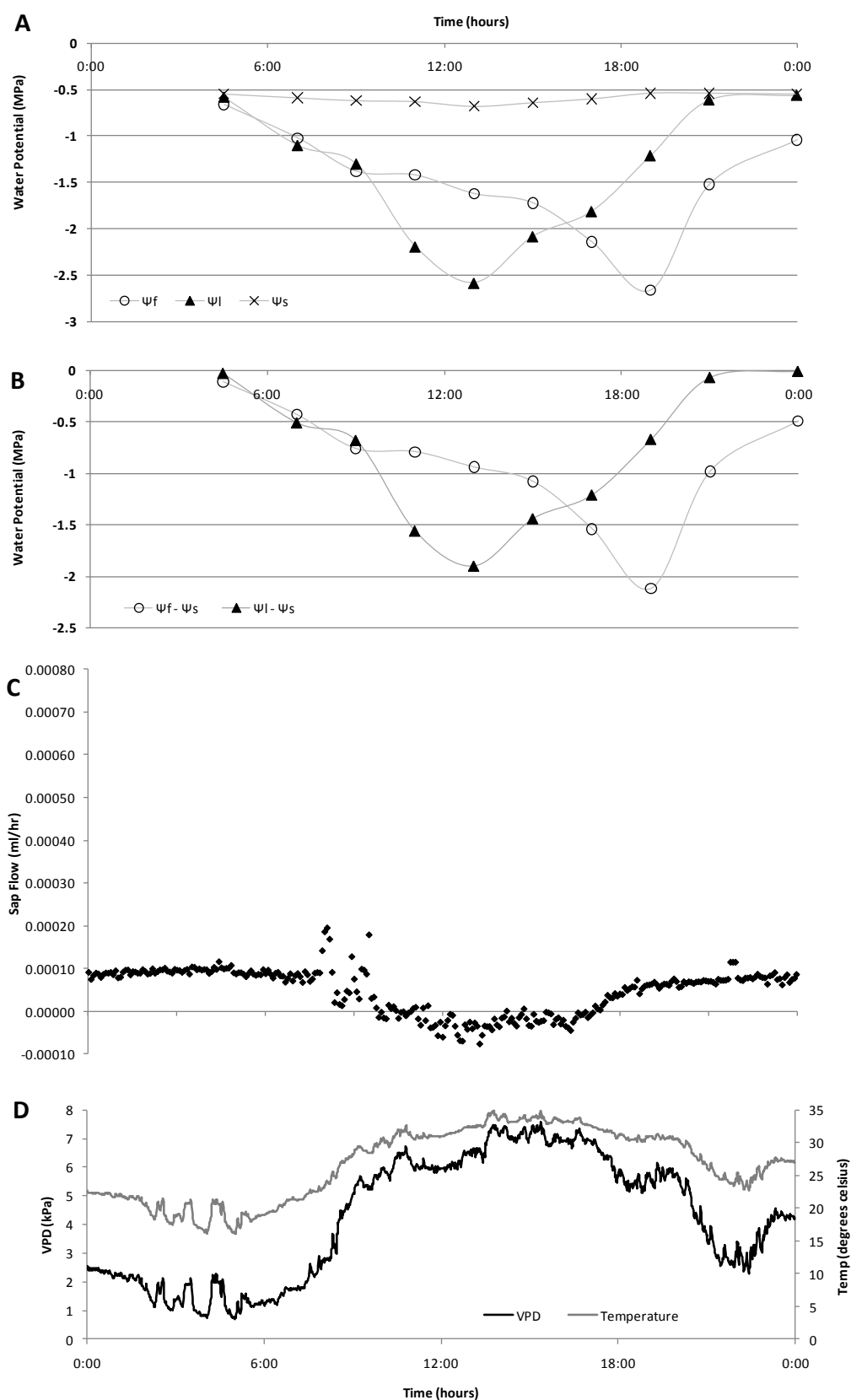


Figure 17 Diurnal changes in; (A) water potential of fruit, leaves and branch xylem, (B) potential gradients between fruit and xylem and leaves and xylem, (C) Fruit sap flow and (D) Ambient Vapour Pressure Deficit and Temperature on Day 1, the 5th day of January 2008.

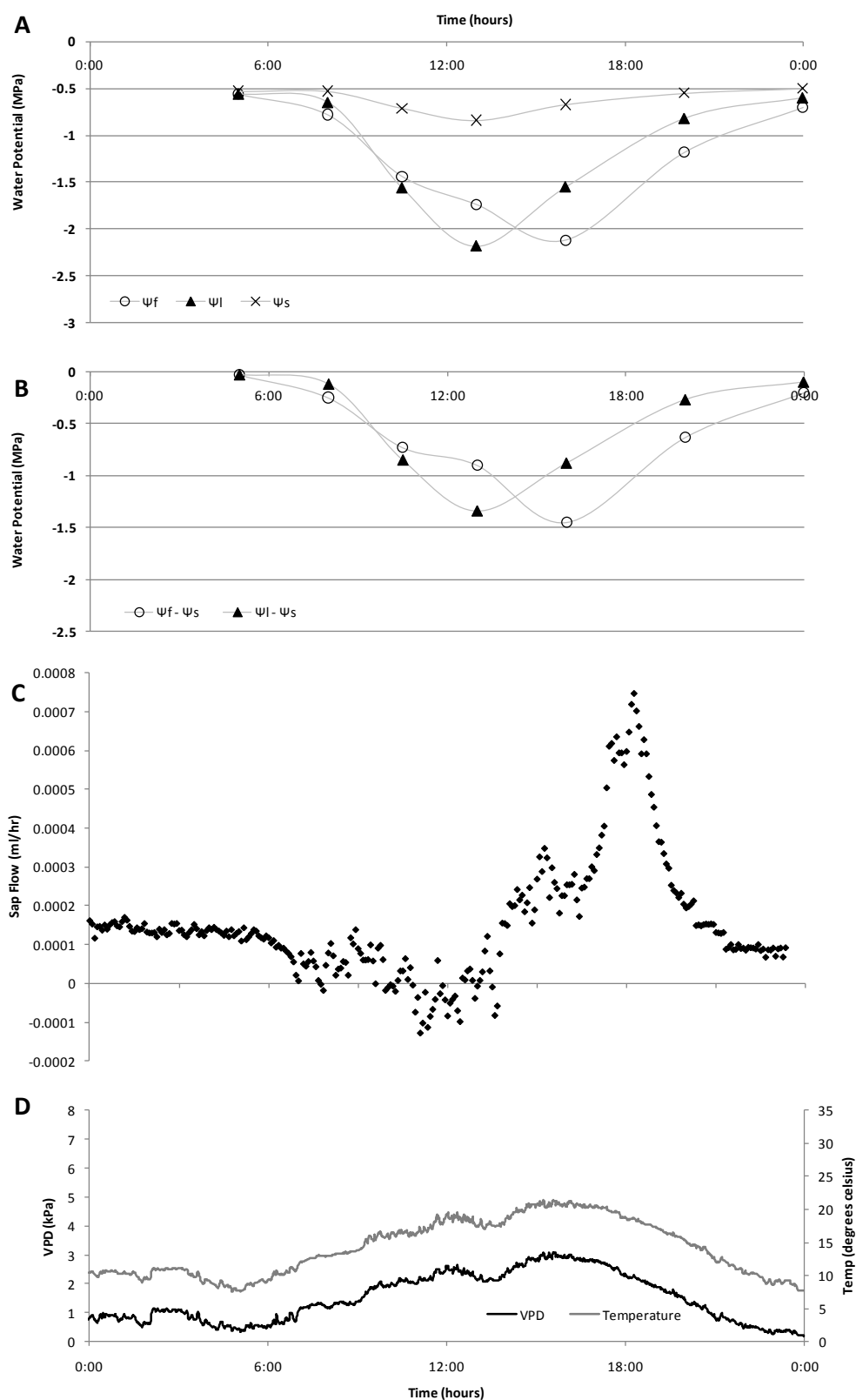


Figure 18 Diurnal changes in; (A) water potential of fruit, leaves and branch xylem, (B) potential gradients between fruit and xylem and leaves and xylem, (C) Fruit sap flow and (D) Ambient Vapour Pressure Deficit and Temperature on Day 2, the 14th day of January 2008.

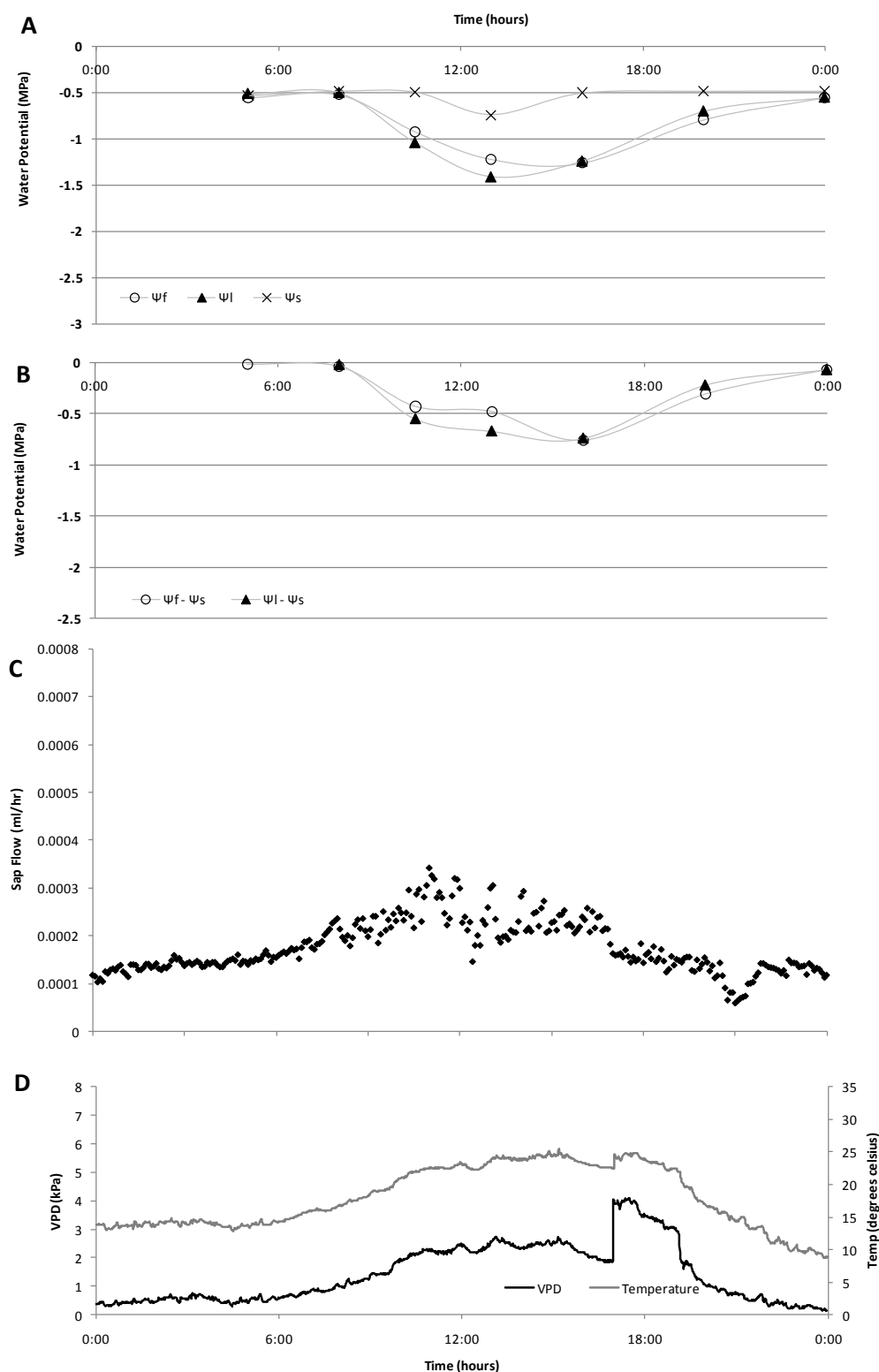


Figure 19 Diurnal changes in; (A) water potential of fruit, leaves and branch xylem, (B) potential gradients between fruit and xylem and leaves and xylem, (C) Fruit sap flow and (D) Ambient Vapour Pressure Deficit and Temperature on Day 3, the 19th day of January 2008.

Both water potential and sap flow values from the targeted days of monitoring diurnal water potential and sap flow patterns with climatic conditions (5th Jan, 14th Jan and 19th Jan 2008) are presented sequentially in Figure 17, Figure 18 and Figure 19. Within the critical preharvest period for sweet cherry cracking for season 2007/08 only four rainfall days were experienced, totalling 5 mm rainfall (see Chapter 4). Day 1 was a hot, sunny, dry day without rain, reaching a maximum temperature of 35 °C. Day 2 was a mild day, sunny in the morning, reaching a maximum temperature of 25 °C and Day 3 also reached a maximum temperature of 25 °C, but was overcast for the entire day, and experienced 1.8 mm of rain during the afternoon.

Water potential of fruit and leaves varied between the three days. Day 1 experienced the greatest VPD (> 7 kPa) and induced the most negative figures potential for both fruit (Ψ_F) and leaves (Ψ_L). The greatest deficit potential (-2.7 MPa) in fruit occurred late in the day (after 6 pm), later than the greatest deficit potential of the leaf. Also, at this time the greatest potential gradient, between the fruit and spur xylem water potential ($\Psi_F - \Psi_S$), existed which exceeded the gradient between leaf and xylem ($\Psi_L - \Psi_S$) by 0.5 MPa (Figure 17B). Day 2, showed a smaller but similar lag in fruit potential development, and similar patterns of potential gradients (Figure 18B). Day 3 experienced less negative overall potential figures, and showed little difference in potentials between fruit and leaves, and between the gradients developed with spur xylem water potential gradients (Figure 19B). VPD on days 2 and 3 did not exceed 3 kPa. Over all three days, xylem potentials showed similar diurnal patterns and fell within a similar range of -0.5 to -0.8 MPa.

Over the three days a significant relationship was found between net sap influx to the fruit (Figure 17C, Figure 18C, Figure 19C) and the potential gradient of leaf and spur xylem ($\Psi_L - \Psi_S$) ($R^2 = 0.64$), but not between influx and the potential gradient of fruit and spur xylem ($\Psi_F - \Psi_S$) (Figure 20).

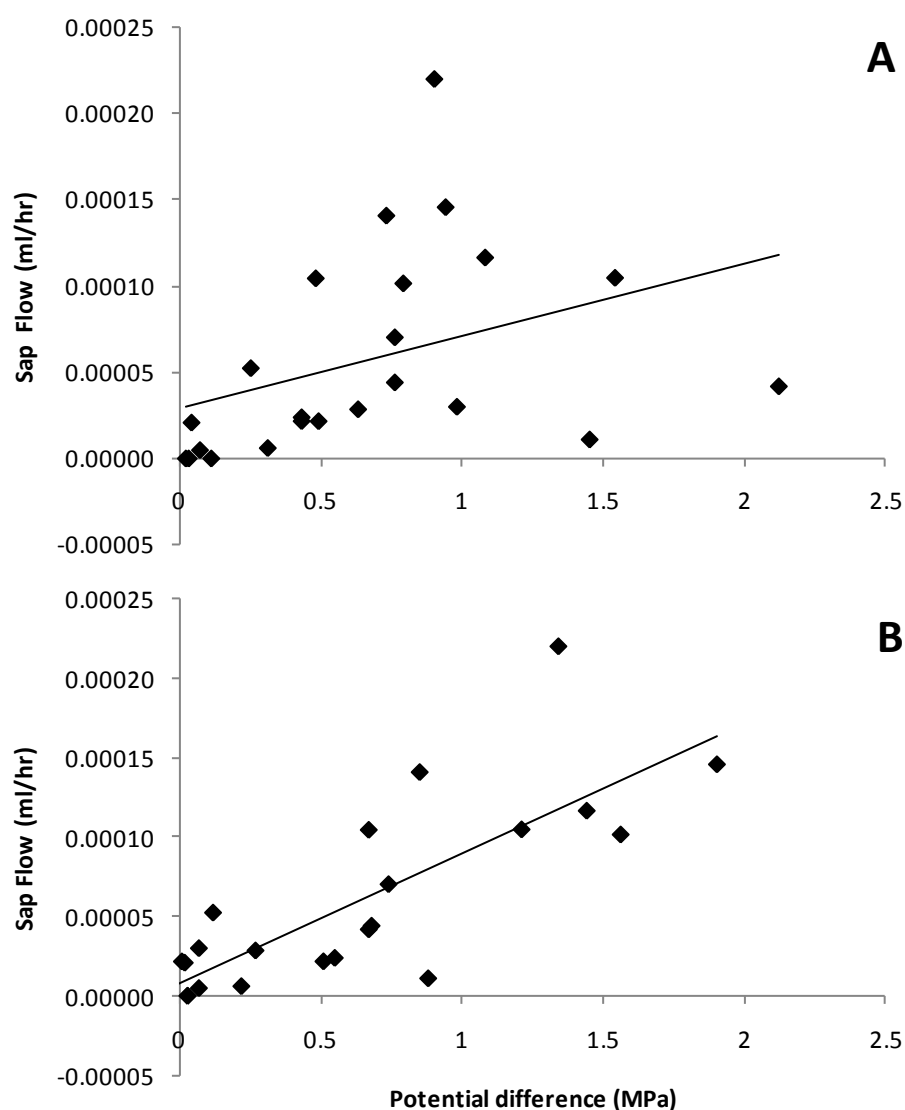


Figure 20 Relationship between (A) absolute potential gradient ($\Psi_F - \Psi_S$) and sap flow ($R^2 = 0.15$), and (B) potential gradient ($\Psi_L - \Psi_S$) and sap flow ($R^2 = 0.64$). Each point represents potential gradient from mean fruit, leaf and spur xylem water potential values ($n = 5$) and mean fruit sap flow ($n = 3$) at a given time.

Influence of Afternoon Rainfall on Diurnal Fruit Water Status

There was a significant difference in the development of turgor and osmotic potentials during the afternoon on the wet day compared to the dry day monitored. Under both dry and wet conditions turgor was high in the morning but decreased by 1.30 pm, with the lowest turgor experienced by fruit under wet conditions, just following the onset of rainfall. This corresponded with an increased osmotic potential. Late in the day, this trend had reversed with fruit under wet conditions experiencing the highest turgor, but also the lowest osmotic potential (Figure 21).

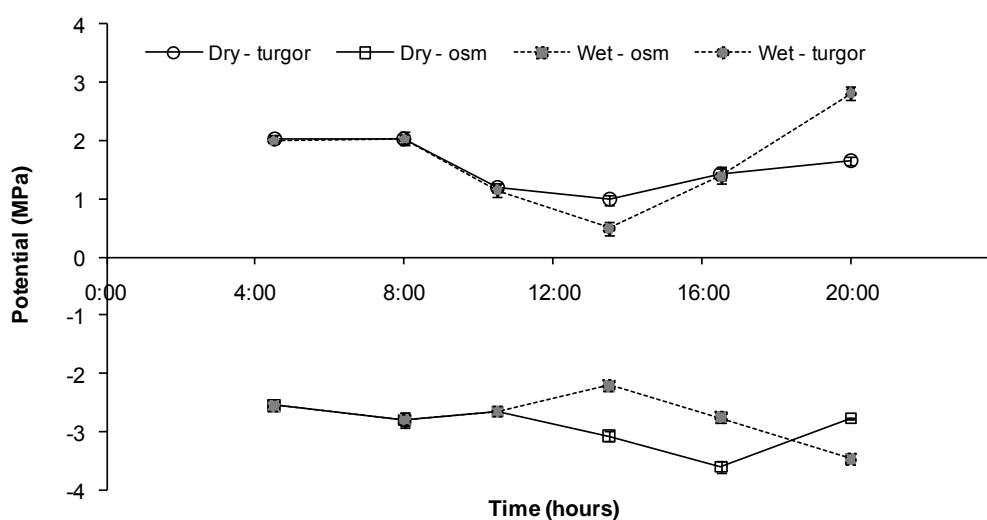


Figure 21 Mean turgor and osmotic potential of fruit ($n = 5$) under wet and dry conditions. Error bars indicate the standard error of the mean (SEM).

Discussion

Increased incidence of vascular induced or „side’ cracking in sweet cherry with simulated rainfall occurring during the afternoon, and an increased spike of vascular influx to the fruit following rainfall was shown in Chapter 5. Results from this study (Chapter 6) support these findings; a greater demand for available water during the afternoon was evident through increased potential gradients, and simulated afternoon rainfall resulted in increased fruit turgor and a corresponding decreased osmotic potential. The trends in this study support a role for potential driven flow rather than purely osmotic driven flow in the development of fruit cracking. The development of a high water potential gradient between cherry fruit and leaves on dry days compared to wet days is consistent with environmental influences on evaporative demand for water flow through the tree and such gradients have been noted in a study of French prune (*Prunus domestica* L.) (Milad and Shackel 1992).

The significant likelihood of influx with rainfall across all seasons further confirms vascular supply of water to the fruit following rainfall. Comparative sap flow magnitudes between days monitored fit expectations. The strong association of influx to the fruit with leaf water status implies that leaf evaporative demand was the dominant driver of flow within the spur/fruit/leaf complex. The stem-leaf pathway has been identified as offering the least resistance to flow (Oyarzún *et al.* 2008). The significant relationship between the potential gradient of leaf and spur xylem water potential with the absolute magnitude of sap flow through the fruit pedicel gives strong support to this claim by demonstrating the indirect influence of environmental factors on evaporative demand. Increased diurnal fluctuations in

potential gradients responding to changes in environmental conditions may, therefore, increase diurnal fluctuations in fruit growth patterns. Oyarzún *et al.* (2008) showed that sweet cherry fruit trees do not have a significant water storage component (i.e. the fruit) which will not only affect total tree hydraulic conductivity, but also lead to periods of shrinkage during periods of high evaporative demand. These extreme fluctuations in shrinkage and expansion resulted in higher cracking levels in bell pepper (Yao *et al.* 2000).

A whole tree approach to understanding vascular flow in sweet cherry would advance the investigation of flow at the spur level. Results of this study show that flow is closely linked to ambient vapour pressure deficits, and it has been previously shown that high VPD's have induced lowered leaf water potentials in carrots, even in well watered plants (Gibberd *et al.* 2000). Light-induced leaf transpiration has resulted in water efflux from tomato fruit (Ohta *et al.* 1998), and efflux has been observed in the present study in response to evaporative demand by the leaf. Fruit efflux was only seen on dry days; when a greater potential gradient between leaf and spur xylem ($\Psi_L - \Psi_S$) than fruit and spur xylem ($\Psi_F - \Psi_S$) existed (Days 1 & 2). Influx was restored when the gradients were reversed supporting further that excess supply of water to the fruit, and subsequent cracking can be vascular-induced (as was implicated by the results of Chapter 5). Cherries have relatively few stomata (Glenn and Poovaiah 1989) and are therefore less likely to be efficient regulators of turgor and more likely to suffer from cracking when supplied with excess vascular water.

This study supports the hypothesis put forward by Sekse (1995b) who suggested the rate of internal water supply increased cracking incidence and the findings of

Chapter 5 of this thesis, which suggested a close leaf interaction in the development of vascular induced „side’ cracking. Furthermore, the development of cracks in tomato has been linked to environmental conditions which favour rapid growth; times of increased sap influx and subsequent increased growth rates (Guichard *et al.* 2001).

Results indicate that influx to the fruit following rainfall may be potentially driven, and dependant on leaf interactions with evaporative demand of the ambient environment. It also shows that excess water responsible for cracking may be delivered according to normal growth and expansion patterns, and that fruit are not efficient self regulators of turgor. These results strengthen the suggestion put forward in Chapter 5 that cracking in cherry fruit following rainfall can be vascular-induced. Diurnal influx to the fruit through either the xylem and phloem were not explored however, given the results presented here, the separation and contribution to the vascular supply of excess water to the fruit following rainfall is warranted.

Conclusion

This study highlights the importance of understanding normal cherry fruit growth and dry matter accumulation, especially in response to water fluxes. The impact of cherry tree source:sink relationships on these fluxes and the involvement of crop load and fruit properties on the development of vascular induced cherry fruit cracking is a justified avenue of investigation.

Chapter 7

Crop Load and Fruit Cracking in Sweet Cherry (*Prunus avium* L.)

This chapter explores the relationship between cherry fruit and skin properties and the level and type of cracks that develop. The relationship between cracking and crop load of the tree was also investigated. Crop load was included because of the effect on fruit growth through competition for photoassimilate. Chapter 7 addresses the research objectives;

- To examine the relationships of fruit and skin properties with cracking incidence
- To examine the relationship of crop load with cracking incidence

Introduction

Cherry fruit size and quality is an important factor in production and sales of sweet cherry fruit (Proebsting and Mills 1981). Sweet cherry trees are typically upright, vigorous and non-precocious (Lang *et al.* 2004) so orchard management practices focus on achieving high yields of premium quality fruit through balancing reproductive and vegetative growth. Manipulation of the number of fruit (crop load) on the tree, and leaf area, can be used to encourage larger and sweeter fruit through balanced carbohydrate supply and demand (Lang *et al.* 2004, Whiting and Lang 2004, Spayd *et al.* 1986). However in many of these studies, yield losses due to cracking have not been presented (Proebsting and Mills 1981) even when the economic losses due to cracking can be significant (Hanson and Proebsting 1996). Given the findings in Chapter 5 and 6, that cracking can be induced by internal vascular flow, it is posited that higher crop loads will reduce the incidence of cracking through increased competition between fruit for assimilate supply and lower daily growth rates.

It has been hypothesised that higher crop loads increase competition between fruit for carbohydrates and that lower crop loads result in higher assimilate supply for individual fruit (Spayd *et al.* 1986), and that there can be a resultant increase in size (Spayd *et al.* 1986) and concentration of sugars (Proebsting and Mills 1981). It has also been noted however, that lower crop loads are associated with increased vegetative growth (Kappel 1991) and that current season's vegetative growth is a strong sink for carbohydrates. Richardson (1998) conceded that diurnal translocation of sugars from leaves to fruit can be variable, and therefore assessing relationships between sugars and cracking are difficult.

Cherry fruit are strong sinks (Ayala and Lang 2008) and it has been noted that removal of spur leaves had little effect on fruit quality because alternative supplies of carbohydrates were sourced (Whiting and Lang 2004). Fruit and leaf ratio can be manipulated for optimum quality. Two flower buds per spur has been suggested as the ideal based on research in the USA (Whiting and Lang 2004). Interaction between fruit and leaves was also implicated in the development of cracking in Chapter 5 that showed leaf removal decreased the incidence of internally supplied, vascular driven side cracks in cherry fruit. The possible causes for this are further explored in Chapter 6 where diurnal potential gradients and evaporative demands on the leaf influence vascular flow to the fruit imply a local fruit and leaf interaction.

Thus, given that fruit size (Simon 2006) and sugar levels (Christensen 1996) have been associated with the development of cracking, the effect of crop load on fruit cracking warrants investigation. The aim of this present study is to further investigate both the relationship of fruit properties with cracking, and the relationship between crop load and cracking.

Materials and Methods

Plant Material

Mature trees, grown on F12/1 rootstock were used in all field trials. Trials were undertaken from late October to late January during seasons 2005/06, 2006/07 and 2007/08, in two commercial orchards in Huonville and Bushy Park, Tasmania (Australia) (42°98'S, 177°08'E and 42°71'S, 146°90'E respectively). Both orchards were subjected to standard industry management practices. To investigate the effect of crop load on fruit cracking and type three manipulated crop load trials (Trials 1, 2 and 3 in seasons 2005/06, 2006/07 and 2007/08 respectively) were undertaken and a survey of natural crop load and fruit properties over the three years was performed. In addition to this the relationship between levels of cracking *in situ* and the cracking index was evaluated.

Manipulated Crop Load Trials

To assess the impact of crop load on crack development, manipulated crop load trials were undertaken on variety „Simone’ in 2005/06 (Trail 1) and 2006/07 (Trial 2) at Huonville. In each trial, treatments were applied at pit-hardening (Stage II of fruit growth and development). Treatments included a low, medium and high crop load, which aimed for 2, 5 or 8 fruit per cm² trunk cross sectional area (TCSA) respectively. Where the high crop load specified could not be reached natural crop load was determined and used. Prior to treatment in all trials, trees were blocked into groups based on trunk girth. There was a minimum buffer of two untreated

trees between treatments. Three replicates were used in Trials 1 and 2 (whole tree plots).

An expanded manipulated crop load trial (Trial 3), as described above, was undertaken in 2007/08 at Bushy Park, due to the higher fruit set evident at this orchard. Treatments included a low, medium and high crop load, which aimed for 2, 5 or 8 fruit per cm² branch cross-sectional areas (BCSA) respectively, with two treatment application times; at pit-hardening (early) and at three weeks prior to harvest maturity (late). Five replicates were used in Trial 3, but in contrast to Trials 1 and 2 each treatment was applied to single branches (branch plots) in a randomised complete block design.

At harvest, when all fruit from all trial trees were harvested, cracking incidence was determined for all manipulated crop load trials, and in Trial 3 weight, diameter and total soluble solids were also measured from 100 randomly selected non-cracked, blemish-free fruit from each treatment. In all manipulated crop load trials, the actual crop load achieved for all trial trees was recorded at harvest.

Natural Crop Load and Fruit Properties Survey

Natural crop load was recorded at harvest in all three seasons for three randomly selected whole trees of available varieties („Kordia’, „Lapins’, „Regina’, „Simone’, „Sweetheart’, „Sylvia’ and „Van’; „Lapins’, „Simone’ and „Van’ were not determined in 2007/08, 2006/07 and 2005/06 respectively). All fruit were harvested and cracking levels recorded. Natural crop load was used to assess the relationship with the cracking incidence recorded at harvest.

In all three trial seasons, non-cracked blemish free fruit from each variety was grouped, and a sub sample of 30 non-cracked fruit was taken and each fruit was assessed for size, weight, total soluble solids, stem length and skin thickness. Mean fruit property values were used to assess the relationship with the incidence of cracking *in situ* for each variety. In 2007/08, varieties „Sylvia’ and „Van’ were chosen to investigate skin properties due to the difference in crack type susceptibility of each variety as found in Chapter 4. For each variety, skin mechanical properties in both the radial and longitudinal planes were examined.

In 2007/08, fruit were additionally assessed for cracking (using 50 non-cracked fruit per variety) with the cracking index immersion method developed by Verner and Blodget (1931) as cited in and refined by Christensen (1972). The relationship between the cracking index values and the level of cracking recorded in the field was investigated. In conjunction with assessing cracking index for each variety, an additional subsample of 50 non-cracked fruit from variety Simone were tested for cracking incidence after immersion in water as per the cracking index method, with either stems removed or left intact.

Measurements

Cracking incidence in all trials was determined as per Chapter 3, with apical-end cracks and stem-end cracks combined to give a level of cuticular cracks. Chapter 5 concluded that these crack types were likely to be induced through the same mode of water uptake.

All fruit for assessments were harvested between 7am and 12 noon and cracking assessments, morphological measurements and laboratory-based measurements were undertaken on the same day as harvest. Climate data for the months preceding and during harvest was obtained from the Australian Bureau of Meteorology Stations at Grove and Bushy Park (situated less than 5 km from each trial site).

Determination of Crop Load

Prior to treatment application in manipulated crop load trials, tree or branch girth circumference was measured in centimetres at a point 5cm above the graft union or above the trunk/branch junction. TCSA or BCSA was calculated for each tree for the area (A) of a circle using the formula ($A = C^2/4\pi$), where C = circumference (cm) as described in Chapter 3. Crop load was determined as total fruit number per TCSA or BCSA. To determine natural crop load, all fruit were counted and crop load expressed as number of fruit per TCSA.

Fruit Property Tests

Fruit size, weight and total soluble solid (TSS) concentration (brix^o) were measured as described in Chapter 3 (page 58-60). Stem length (mm) was measured using Vernier callipers and skin thickness was recorded microscopically using a Nikon SMZ800 dissecting microscope.

Skin Property Tests

Skin properties were measured using a Series 5500 Instron Materials Testing Machine (Instron Inc.) fitted with Series 2701 Pneumatic Grip Controls, with skin properties computed using software program MERLIN. Ten individual fruit replicates were used for each variety in each plane. To avoid tissue dehydration skin strips were prepared individually, immediately prior to testing. Skin was carefully excised from one side of each fruit sample using a scalpel, from which a 5mm x 20mm strip was cut. Skin strips were held in position with clamps, with the original length of skin between clamps being 10 mm. A pre load of 0.1N was applied, then extension of tissue occurred at a speed of 1.0 mm.min⁻¹ until breaking point was reached. Maximum tensile stress (MPa) was recorded at tissue failure (breaking point). Extensibility of the tissue was defined as the maximum strain (ϵ) at which breakage occurred, Strain was calculated from the change in length of the tissue as;

$$\epsilon = (L - L_0) / L_0$$

where L is the final length and L₀ is the length at time 0.

Statistical Analyses

To assess the relationships between crop load and cracking incidence, and crop load with mean fruit properties, data were subject to linear regression tests. To assess the effect of crop load on fruit property after accounting for variety, mean fruit property data were subject to ANOVA using variety as a fixed factor and then to ANCOVA (crop load as the covariate) using PROC GLM (SPSS version

17). GenStat version 12.1 was used to perform ANOVA to determine the impact of crop load and timing on fruit properties for Trial 3. Unless specified, all results quoted as ‘significant’ are at probability level of 0.05.

Results

Manipulated Crop Load Trials

A negative linear relationship between crop load and cracking incidence was recorded in both seasons 2005/06 ($R^2 = 0.863$, $P < 0.001$) and 2006/07 ($R^2 = 0.709$, $P = 0.004$) (Figure 22). A negative linear relationship was also recorded between crop load and cuticular cracking in 2005/06 ($R^2 = 0.903$, $P < 0.001$) and 2006/07 ($R^2 = 0.511$, $P = 0.03$), and between crop load and side cracking in 2006/07 ($R^2 = 0.575$, $P = 0.02$). The effect of crop load on cracking incidence was greater in season 2005/06 than 2006/07, as indicated by the significantly greater magnitude of the slope (Table 6). In the three weeks prior to harvest of „Simone’ there was a similar amount of rainfall in both trials (Trial 1 in 2005/06 experienced 37 mm rainfall and Trial 2 in 2006/07 experienced 41 mm rainfall). Only 1 mm rainfall was experienced in 2007/08 (Trial 3). Negligible cracking (less than 1%) occurred during Trial 3; there was no significant interaction between timing and level of crop load applied and no significant main effects on cracking incidence (data not shown).

There was no significant relationship between actual crop load and mean fruit weight in either of Trials 1 or 2. For Trial 3, there was no significant effect of timing (early or late) or level of crop load applied (low, medium or high), or their interaction on either fruit weight or diameter. There was a significant (< 0.001) interaction between timing and level of crop load applied for fruit total soluble solids, the late timing reducing the range of brix values as follows; for early application of crop load treatments low, medium and high the TSS values were

25.28, 25.45 and 21.85, and for the late application values were 24.09, 24.30 and 22.48.

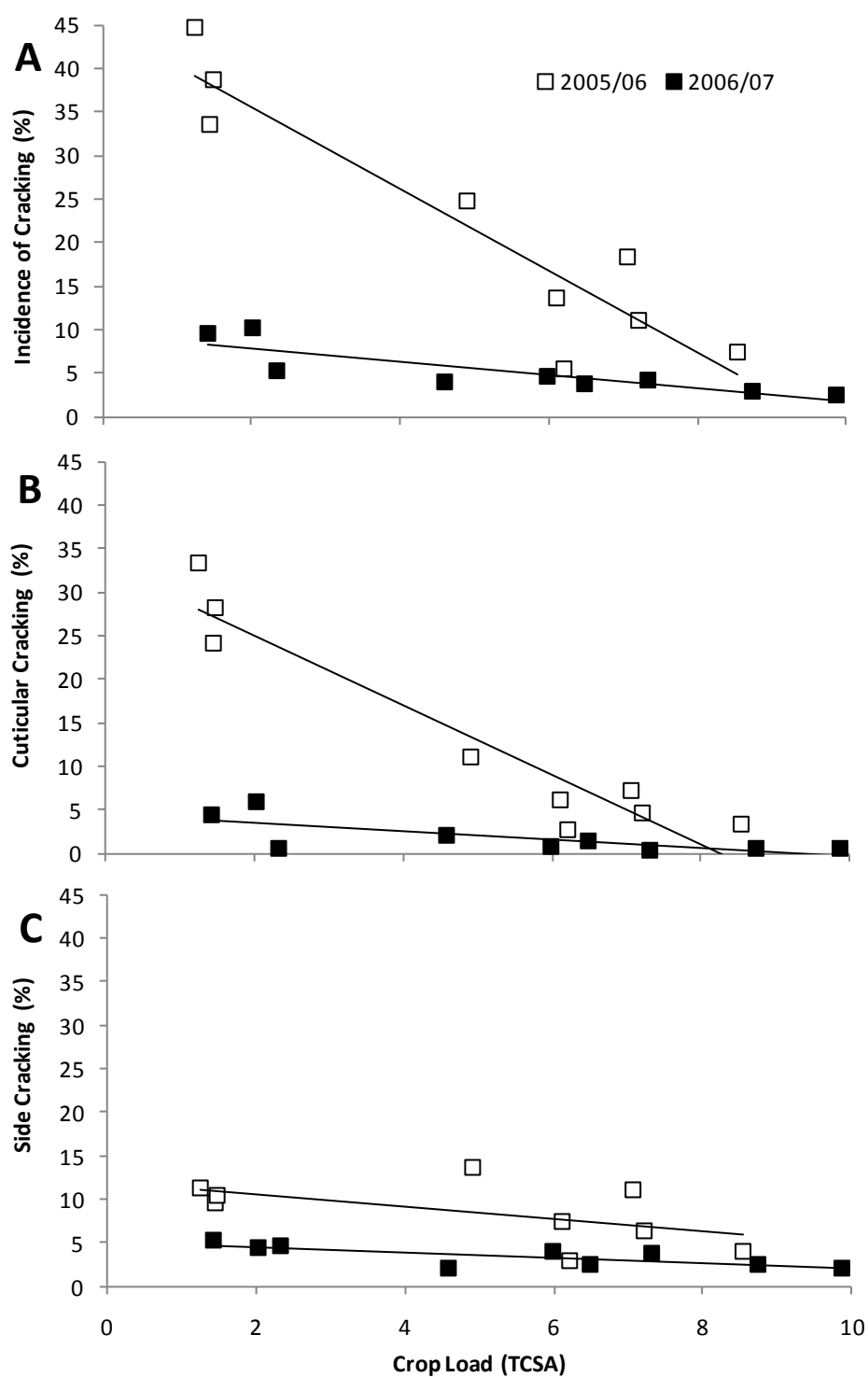


Figure 22 Percentage of total cracked fruit (A), cuticular-cracked fruit (B) and side-cracked fruit (C) with manipulated crop load (TCSA). Each point is for an individual tree. A significant relationship was found between crop load and total cracking incidence in 2005/06 and 2006/07, and between crop load and cuticular cracking in 2005/06 and 2006/07, and between crop load and side cracking in 2005/06. A significant difference between seasons was found for total cracking and cuticular cracking.

Table 6 Slope (B) of the linear regressions with 95 % confidence intervals (CI) calculated for crop load and cracking incidence. Values followed by different letters indicate a significant difference ($P < 0.05$).

Crack type	Season	Slope (B)	CI
Total	2005/06	-4.69 ^a	± 1.41
	2006/07	-0.77 ^b	± 0.37
Cuticular	2005/06	-4.69 ^a	± 1.41
	2006/07	-0.77 ^b	± 0.37
Side	2005/06	-0.69 ^a	± 0.81
	2006/07	-0.30 ^b	± 0.20

Natural Crop Load and Fruit Property Trials

Reduced crop loads recorded higher levels of cracking incidence (Figure 23). Cracking incidence remained low (less than 5%) for crop loads higher than ten fruit per cm² TCSCA in all years and for all crack types (Figure 23). When only using data points less than 10 fruit per cm² TCSCA, relationships between cracking and crop load were found to be significant for seasons for all crack types in 2005/06 (total, $R^2 = 0.893$, $P < 0.001$; cuticular, $R^2 = 0.853$, $P < 0.001$; side, $R^2 = 0.540$, $P = 0.006$) and for total and cuticular cracks in 2006/07 (total, $R^2 = 0.576$, $P = 0.005$, cuticular, $R^2 = 0.463$, $P = 0.03$).

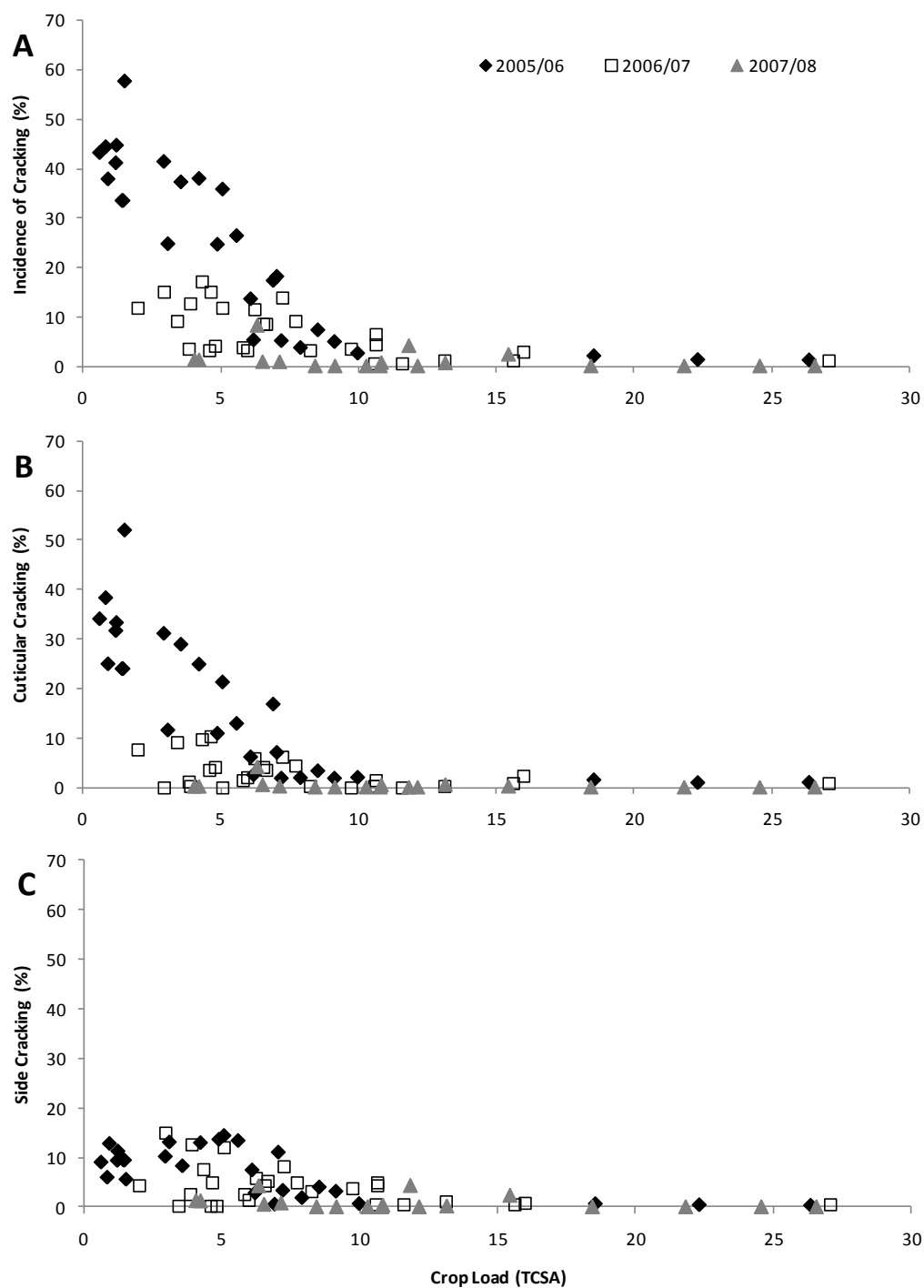


Figure 23 The percentage of total cracked fruit (A), cuticular-cracked fruit (B) and side-cracked fruit (C) with natural crop load (TCSA). Each point is for an individual tree. A significant relationship was found between crop load and total cracking incidence in 2005/06 and 2006/07, and between crop load and cuticular cracking in 2005/06, and between crop load and side cracking in 2005/06 and 2006/07.

Fruit properties recorded were correlated independent of variety and season. A significant ($P < 0.001$) correlation was found between fruit weight and diameter, fruit weight and skin thickness, and fruit diameter and skin thickness (Table 7). A significant ($P < 0.05$) positive correlation was also found between stem length and fruit total soluble solids (Table 7). No relationship was found between either fruit weight or diameter with total soluble solids (Table 7). Mean values of fruit properties in reference to the mean cracking levels were determined from Trial 4. Across all varieties and seasons, no significant relationship was found between any of the fruit properties values with total cracking incidence or individual crack type incidence. Mean fruit weight and total soluble solids within each variety and season are presented in Figure 24, along with mean crop load levels and cracking incidence. There is a distinct trend between increased crop load and decreased crack levels. Little difference can be observed between either weight or total soluble solids and changes in crop load, except perhaps a slight trend in variety „Sylvia’ where a decrease in weight, but not total soluble solids, occurs with a dramatic increase in crop load in the third season, 2007/08. A significant variety effect was found on fruit weight ($P = 0.02$), total soluble solids ($P = 0.001$) and stem length ($P < 0.001$). After accounting for variety, and analysing data using crop load as a covariate, a significant effect of crop load was found for fruit weight only ($P = 0.33$).

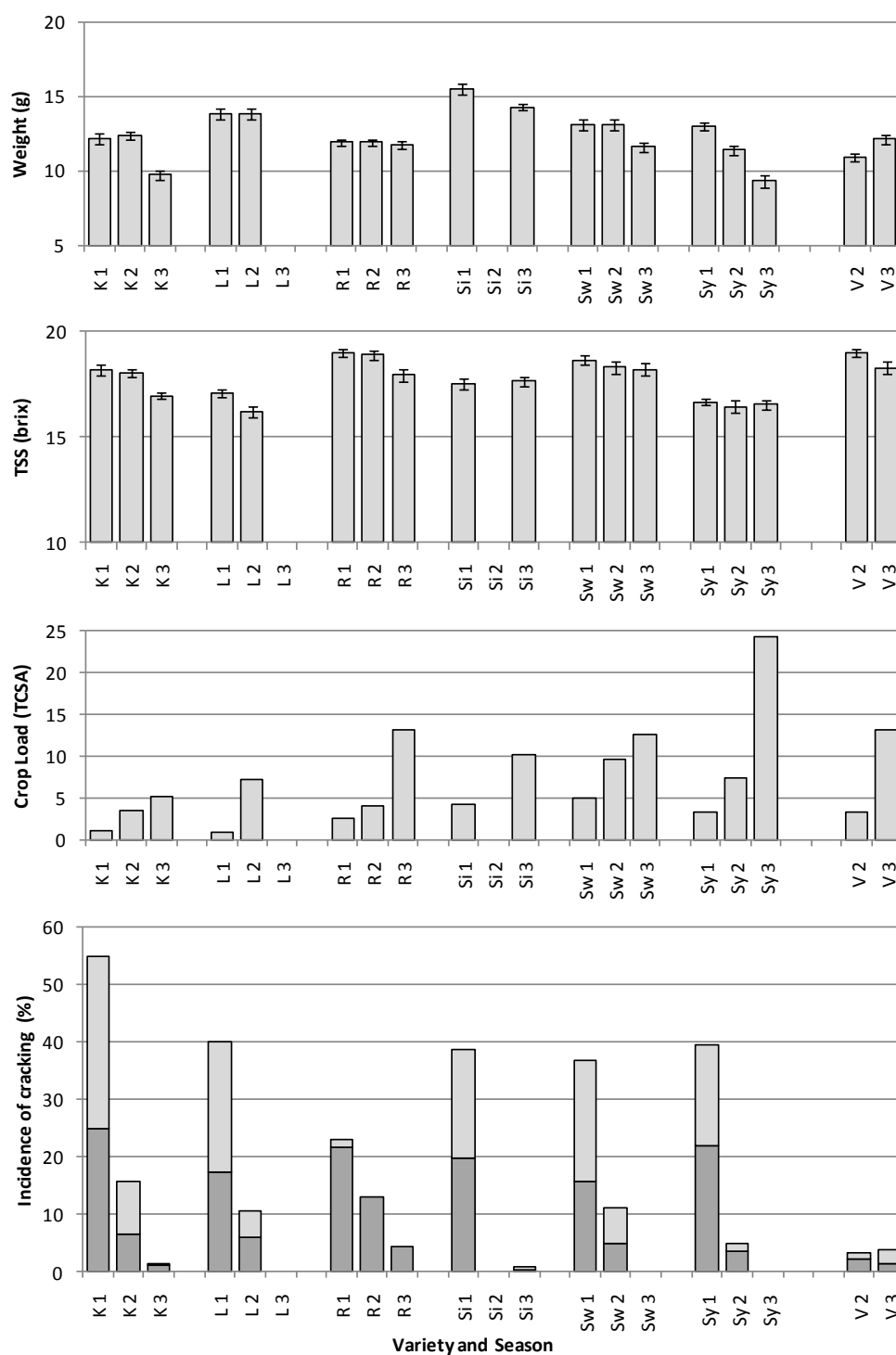


Figure 24 Mean fruit weight and total soluble solids for seven varieties over three seasons (error bars represent SEM, $n = 30$) with mean crop load levels ($n = 3$) and cracking incidence for the same varieties and seasons. For cracking incidence, expressed as percentage of total yield, columns represent total incidence, where light areas represent incidence of cuticular-cracked fruit, and dark areas represent incidence of side-cracked fruit. Horizontal axis labels represent variety by letter (K – Kordia, L – Lapins, R – Regina, Si – Simone, Sw – Sweetheart, Sy – Sylvia, V – Van) and season by number (1 – 2005/06, 2 – 2006/07, 3 – 2007/08).

Table 7 Correlation between fruit properties measured (diameter, weight, total soluble solids, stem length and skin thickness) from individual fruit from nine varieties over three years (N=440).

		Weight	TSS	Stem	Skin
Diameter	Pearson Correlation	0.895	ns	ns	0.170
	Sig. (2-tailed)	<0.001			<0.001
Weight	Pearson Correlation		ns	ns	0.181
	Sig. (2-tailed)				<0.001
TSS	Pearson Correlation			0.095	ns
	Sig. (2-tailed)			0.046	
Stem	Pearson Correlation				0.102
	Sig. (2-tailed)				0.032

No significant difference in Strain (ϵ) or Load (N) at tissue failure was seen between the radial and longitudinal planes of tissue in variety „Sylvia’. Variety „Van’ exhibited a significant ($P < 0.001$) difference between planes in both Strain (ϵ) and Load (N) at tissue failure (Figure 25). A significant ($P < 0.001$) difference between varieties was found for strain in the radial plane, and for Load at tissue failure in both the radial and longitudinal planes.

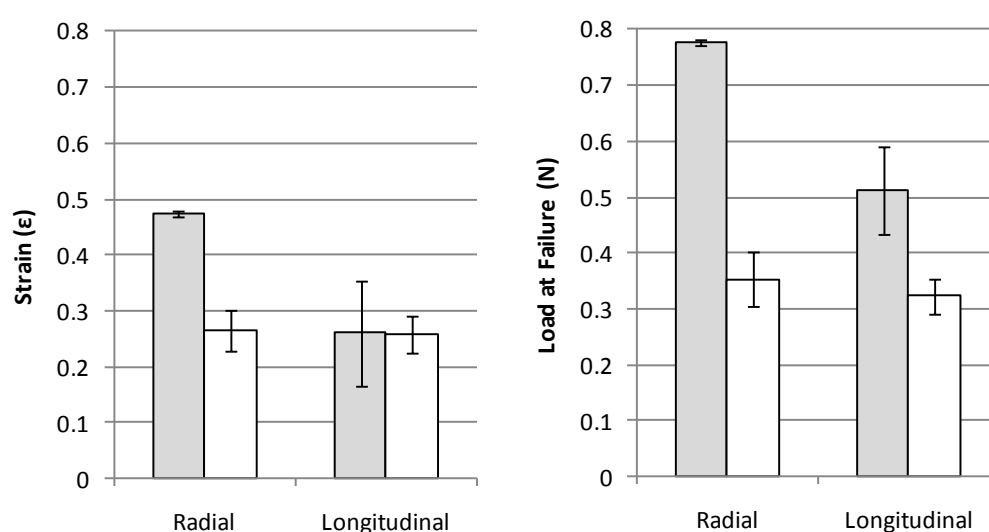


Figure 25 Mean fruit skin Strain (ϵ) and Load (N) at tissue failure in both the radial and longitudinal planes in Variety ‘Van’ (grey columns) and ‘Sylvia’ (white columns). A significant ($P < 0.001$) difference was found between planes in variety ‘Van’. Error bars represent SEM, $n = 10$.

While cracking index values for eight varieties (Table 8) are of a different magnitude to actual cracking, a significant correlation with total cracking incidence ($P = 0.011$) and incidence of side-cracked fruit ($P < 0.001$) was found. No significant relationship was found between cracking index and incidence of cuticular cracked fruit. When indices determined in this study were compared with indices presented for three of the eight varieties in Christensen (1996) and four of the eight varieties in Greco *et al.* (2008), values appear to be vastly

different. Given that this immersion method depends on water uptake across the skin, the impact of stem removal on uptake and subsequent index value was also investigated. No difference was found between values for fruit which had stems removed and fruit with stems attached (both had index values of 25).

Table 8 Cracking index (n = 50) determined for eight varieties in season 2007/08, compared to three and four varieties from Christensen (1996) and Greco *et al.* (2008). Mean incidence of total cracking, side cracking, and cuticular cracking are also given. A Significant correlation was found between cracking index and both total and side –crack incidence.

	2007/08	Cracking index		Cracking incidence		
		Christensen	Greco	Total	Side	Cuticular
Kordia	39	na	na	1.2	1.0	0.2
Regina	64	na	na	4.5	4.4	0.1
Simone	25	na	na	0.8	0.3	0.5
Summit	6	71	17	0.0	0.0	0.0
Sunburst	17	57	13	0.0	0.0	0.0
Sweetheart	6	na	na	0.0	0.0	0.0
Sylvia	6	na	42	0.0	0.0	0.0
Van	23	74	15	3.7	1.4	2.3

Discussion

Lower crop loads resulted in greater incidence of fruit cracking in sweet cherry. This was seen with both natural crop loads in the field and manipulated crop loads and was a consistent trend regardless of crack type. In manipulated trials, the difference in magnitude of cracking between 2005/06 and 2007/08 confirm a strong seasonal impact on crack development as shown by the results of Chapter 4. The timing of fruit thinning also implies that the effect of crop load on final cracking levels are determined post cell division, and more likely to be attributable to cell expansion. This supports the findings of Yamaguchi *et al.* (2002) that linked cracking susceptibility to cell length.

A strong seasonal influence was also noted in the natural crop load survey, where lower crop loads induced a significantly higher proportion of cracked fruit in the years which experienced late season rainfall. The number of cuticular cracked fruit in low cropping trees increased significantly in two seasons. Relative canopy cover on a whole tree basis in low cropping trees, compared to high cropping trees, may prevent fruit from drying in a timely and effective manner, confirming the importance of leaf:fruit ratio in quality management decisions (Whiting and Lang 2004). Cuticular crack formation was also seen to increase with application of surface water; and leaf removal induced a higher incidence of apical end cracks (Chapter 5). Thus fruit exposure from ineffective canopy cover may promote apical-end cracks through surface application of water which persists as a retained droplet in the apical end of fruits, whilst the bulk of the fruit dries with exposure to air circulation and higher light intensity. Again, the influence of season is confirmed and climatic conditions surrounding rainfall events should be recorded

and investigated. In addition, the development of the cuticle during early growth stages should be investigated with regard to cuticle integrity during periods of cracking susceptibility. The number of side cracked fruit in low cropping trees increased significantly only in one season, which recorded more rainfall over the harvesting season than other seasons (Chapter 4, page 79).

This study reports no change in fruit weight with manipulated crop loads, contrary to expectations given the known relationships between crop load and fruit quality (Whiting and Lang 2004, Spayd *et al.* 1986). The results of this present study, however, are not surprising as all crop loads are relatively small, the high load only reaching eight fruit per cm² TCSA.

Variation in fruit properties was mostly influenced by fruit variety, with crop load only further influencing fruit weight, but not size, sugar levels, stem length or skin thickness. Interestingly, across seasons a weak positive relationship between stem length and total soluble sugars was recorded. Stem length may be an indication of functioning vascular pathways or, of adequate resources during fruit development and maturation. However, cracking incidence was not significantly correlated with the fruit properties recorded. In particular, there was no relationship found between fruit size or sugar levels with cracking incidence. This is in contrast with accepted views that both fruit size (Simon 2006) and sugar levels (Christensen 1996) are closely linked with cracking. The findings of Costes *et al.* (2000), who suggested vegetative growth was providing a strong photoassimilate sink in apricots, support these results whereby crop load may not strongly influence source:sink relationships. Results do also confirm the findings of Moing *et al.*

(2004) who reported no difference in sugar levels between varieties of varying cracking susceptibility.

Furthermore, the results of this present study can be explained by the level of crop loads achieved under normal orchard practice. Fruit loads are relatively low in trees from this study; the highest value reaching about 27 fruit per cm² TCSA, or the equivalent of just over 2000 fruit on a tree with a trunk circumference of 30 cm. It is possible that fruit quality (size and sugars) is not diminishing under this scenario as there are still available resources within the tree from which to draw. In other words, crop load could still be increased in Tasmania before any discernable decrease in quality is noted. This finding highlights the strong potential for encouraging good fruit set, and subsequent crop load, as a practical and viable management tool in mitigating yield losses from rain-induced cherry fruit cracking.

The incidence of cracking recorded *in situ* was correlated with the cracking index for those varieties, however, it can be seen that this may not necessarily be a viable tool for predicting cracking susceptibility given the differences found in cracking incidence with seasons (Chapter 4), and with location (Christensen 1996, Greco *et al.* 2008). That the cracking index determined in this study correlated strongly with the incidence of side cracks recorded *in situ* supports the build up of turgor within the fruit as a likely driver of cracking (Sekse 1995b), which can be somewhat mitigated by skin properties. The tangential stress experienced by the skin, as described in Chapter 4, was determined using a combination of fruit properties, and was considered to explain varietal differences in cracking incidence. This study further supports these findings given the different results for

the two varieties tested for skin strain. Only one variety showed a difference between the radial and longitudinal planes tested. The skin from variety „Van’ showed a significant difference in both the strain and the Load experienced at breaking point. Variety „Van’ showed a higher cracking index than „Sylvia’ in this study, but experienced lower tangential stress at which cracking occurred (Chapter 4). This could be due to differences in shape, in agreement with Sawada (1934) that found curvature of the skin related to cracking susceptibility.

Conclusion

This study has confirmed that crop load should be a major consideration in orchard practices with regard to cracking. It has also exposed that Tasmanian cherry trees are not resource limited, promoting crop load management as a viable tool in mitigating rain-induced cracking. The results from this study cannot confirm the relationship between fruit size and sugar with the incidence or cracking, but reiterates the importance of skin properties in crack development.

Chapter 8

Discussion and Industry Summary

Rain-induced fruit cracking in sweet cherry (*Prunus avium* L.) is a complex, integrated problem, involving many contributing factors. Given these considerations, investigations of cracking are not only challenging, but difficult to reproduce in the field, and to provide confidence in research findings. This long term study has provided a unique opportunity to reproduce trials over three seasons, with consistent results. The objectives of the studies within this thesis were to investigate factors with the potential to influence crack development and to ascertain the involvement of vascular water uptake. Areas investigated included the effect of season, variety and fruit and skin properties, the effect of crop load and diurnal drivers of vascular flow within the tree. Novel findings from these studies have strengthened and added to the knowledge relating to the underlying mechanisms of crack development.

The existing theory that direct water movement across the fruit skin results in cracking has been confirmed, and in addition the suggested alternative theory that water uptake via the vascular system results in side cracking has also been confirmed. Vascular water flow can provide sufficient water to increase cracking incidence, and has thus addressed the primary aim of the project in providing a conclusive answer to the question surrounding the involvement of vascular flow. Although this mode has been implicated over many years in the literature, cherry cracking research can now progress with increased confidence to include future management options that mitigate the effects of two modes of water entry rather than one.

Rain-induced Cracking

Cherry cracking in this study has occurred within three weeks of commercial harvest and in conjunction with rainfall events. Cracking can be described as rain-induced, however season rather than actual rainfall amount has emerged as having a strong influence on crack development and incidence. Season has been strongly associated with cracking levels recorded *in situ*, for which there are a number of contributing reasons. Primarily, in addition to rainfall, the environment exerts a strong influence on the condition of the fruit during early growth before entering into the susceptible period, but also on the water fluxes within the tree through potential gradients and the ambient diurnal conditions during the susceptible period.

Cracking and crack type (cuticular and side) incidence can also be influenced by variety, crop load and skin properties. The significance of these findings and possible avenues for further research are explored below.

Water uptake mode and the development of different crack types have been intrinsically linked within the studies undertaken. Several findings (Chapter 4 and Chapter 5) display a degree of separation between the development of side cracks and cuticular cracks. That varieties (all grown on the same rootstock) show a particular disposition for a given crack type suggests that there is an inherent structural difference between varieties that renders it susceptible to either one or the other or both modes of water uptake capable of inducing crack development. This is further supported by the difference between varieties in the ability of the fruit skin to withstand tension.

The development over time of each crack type *in situ* also follows different patterns, cuticular crack development occurring in response to rainfall before side crack development. Exposing fruit (and concurrently disabling effective leaf function) on a spur level also promoted a higher response to rainfall in apical-end cuticular crack development, whereas side crack development was reduced. Again, altering the fruit:leaf ratio by manipulating crop load also saw a varied development of crack types whereas natural variation in crop load impacted on both cuticular and side crack development. The incidence of side cracks, but not cuticular cracks, showed a relationship with the cracking index as determined for varieties in the same year.

The water application trials provide strong evidence that different crack types could be promoted by application of water to different uptake points. These findings are based on three seasons of trials and are significant, as they implicate the need to target future management according to the likely mode of water uptake. Side cracks are promoted by water uptake through the vascular system, such that soil water and subsequent whole tree water fluxes become important, while cuticular cracks are promoted by water uptake across the skin, and as such skin properties and canopy cover become important.

There is now evidence to confirm that the alternative vascular pathway for excess water exists in addition to direct water uptake across the fruit skin, and that this pathway results in cracking following rainfall. The two pathways, the resultant crack type, and the contributing factors to each are discussed.

Cuticular Crack Development

The development of cuticular cracks is associated with direct uptake of water across the fruit skin, however given there was no significant difference in sugar levels between cuticular-cracked and non-cracked fruit the driving force for water movement is not necessarily purely osmotic in nature. This crack type occurs only in specific loci of the fruit but this study did not determine osmotic concentration in these areas.

Results from these studies do however suggest that cuticular structure and skin integrity play a role in the development of cuticular cracking. Cuticular conductance decreases as fruit mature and enlarge due to the non-renewing cuticle having to cover an increased surface area. In addition the curvature of the fruit, possibly influencing the final location that surface water will be held to the fruit surface, may play a role as suggested by Sawada (1934). Certainly the significant difference between strain in the longitudinal and radial planes of fruit skin from variety „Van’, which predominantly experienced cuticular crack development would indicate that fruit shape, and subsequent inability to withstand skin extension in the longitudinal plane and ability to withstand skin extension in the radial plane would increase the likelihood of radial cuticular cracks with fruit expansion, but reduce the likelihood of side cracking. It is therefore important in alleviating possible causal factors for the development of cuticular cracks that early growth periods remain as uniform as possible to avoid large fluctuations in diurnal fruit expansion and shrinkage patterns thereby promoting an elastic skin with good cuticle coverage.

The potential impacts of fruit shape may explain why cuticular cracking occurs in localised areas. Some varieties are particularly prone to apical end cuticular cracking, which could be enhanced by fruit shapes which create a „dimple’ at the apical end in which water droplets can be retained, as was observed for variety „Lapins’. While both apical-end and stem-end cuticular cracks can be promoted by surface wetting, distinct individual differences were recorded in different trials. Afternoon applications of water increased stem-end, but not apical-end cuticular cracks, while removing leaves to expose fruit increased apical-end but not stem-end cuticular cracks of the same variety. The afternoon applications of water could be increasing stem-end cracks through an increased temperature effect at that time of day, or through a weakened cuticle at that point due to higher levels of light reaching the stem end (top) of the fruit leading up to the rainfall event. Apical-end cracks may be increased by leaf removal through an increased air flow around the fruit drying the surface but not removing the droplet from the apical-end depression. This trial was undertaken on relatively low cropping trees; the reduction of cuticular crack development with high crop loads may also be due to increased airflow around fruit, due to lowered fruit:leaf ratio, sufficient to remove all surface water. These results would imply that the duration of surface wetting, as well as fruit shape and skin properties, are all contributing factors to the development of cuticular cracking.

Side Crack Development

The development of side cracks is associated with uptake of water through the vascular system of the tree. There was a significant difference in sugar levels between side-cracked and non-cracked fruit, but it cannot be determined if this was causal or resultant from the cracking process. Given that the relationship between sugar concentration and cracking incidence cannot be fully explained it cannot be assumed that the driving force for vascular water movement involved in crack development was purely osmotic in nature.

Results from these studies suggest that the build up of turgor within the fruit is more likely to occur as a result of potentially driven flow, governed by environmental conditions, fruit and leaf interactions and crop load of the tree. The ambient environment is closely associated with both the water status of fruit and leaves, and the direction of sap flow. Potential gradients arising within the fruit/leaf/spur complex in response to environmental conditions are associated with influx of water to the fruit. The most favourable potential gradient for influx to the fruit following rainfall occurs during the afternoon, particularly when there has been preceding sunshine. This scenario supports a rapid influx, as was observed via sap flow on hot days with afternoon rainfall, and the suggestion of Sekse (1995b) that posited rate of internal flow as determining cracking incidence. In addition, the volume of available vascular water following rainfall water may determine incidence when there are low fruit numbers, given the significant increase in side cracks with lower crop loads.

Side cracks were not related to size of fruit, but shape could have an impact, as for cuticular cracks. The variety „Sylvia’, a side crack prone variety in this study, showed a low threshold for radial strain of the skin compared to „Van’. The level of tangential stress experienced by fruit skin of different varieties was linked to the level of side cracking recorded *in situ*. Variety „Sylvia’ also showed an ability to withstand relatively low levels of tangential stress before cracking occurred, and therefore be less likely to withstand increased turgor build-up from within the fruit. Fruit able to withstand only low tangential stress in seasons with high rainfall such as „Sylvia’ and „Regina’ showed a dramatic increase in side crack development. This further adds support to the vascular pathway involvement as it implies an inability to withstand an increased internal turgor pressure being generated. Thus skin properties become important in the mitigation of side cracking as well as cuticular cracking, even though the uptake mode for each crack type is different

A strong, elastic skin is required to withstand the generation of internal pressure through vascular influx to the fruit. This process however, of vascular influx occurring alternately with efflux as part of the normal growth patterns for fruit may actually promote a weakened skin and cuticle. Indeed there may be an interaction between fluctuations in diurnal fruit expansion and shrinkage patterns early in the season with the ability of the fruit skin to remain intact following rainfall.

Future Research Direction

Rain-induced cracking of sweet cherry fruit can occur as a result of two distinct pathways for excess water uptake. This requires a separate approach in terms of management of influencing factors. It is important to note though, that while varieties may have a particular predisposition for one crack type and will require a variety-targeted strategy, the two modes of uptake are not mutually exclusive and can feasibly occur simultaneously. Long term trends in crack development for each variety grown should therefore be monitored before confidently addressing variety specific cracking management.

The strong influence of season has the potential to alter variety response to rainfall, thus careful monitoring of the season leading into the critical pre-harvest period is advisable. Research with the potential to elucidate the effect of seasonal climate on fruit skin and cuticle deterioration, with particular regard to diurnal growth patterns under different irrigation regimes, would be instrumental in explaining the responses seen within this study.

The finding that increased crop load reduces cracking provides the most promising avenue of future management. However, a target crop load for each variety must first be determined that balances the reduction in cracking with no reduction in fruit size or other quality parameter. Furthermore, in order to successfully achieve this target, the ability to gain a good fruit set, and/or thin to a desired level while not compromising resources is required.

Given that the results of this study indicate that cuticular cracking is enhanced by surface wetting of fruit, the currently available techniques to prevent, or reduce the length of, surface wetting should be reinvestigated with particular regard to this type of crack. New techniques must be developed in order to effectively alleviate the development of side cracks. Crop load is one avenue, as mentioned, but more importantly techniques that reduce, or are able to promote the ability to withstand, the rapid influx of vascular supplied water are needed. Further investigation of whole tree water relations, in particular the contribution of phloem and xylem on this rapid influx, is essential (as per Greenspan *et al.* 1994). An exploration of water uptake zones and patterns by the root system of cherry trees and the effect of irrigation volumes are worthy of further research.

Industry Summary

Cherry fruit cracking is a complex phenomenon which is relatively unpredictable due to the interaction of many contributing factors. This study has investigated many of these factors – climate, season, variety, crack type, orchard practices and fruit and skin properties – and can now add to the existing knowledge of cracking. This knowledge should aid growers when making decisions about orchard management by keeping in mind some of the factors that contribute to cracking in Tasmania. This knowledge also provides a suitable platform from which to further investigate existing and new cracking management techniques.

First and foremost, it is very important to know your variety, and how it responds to rain in your particular region and climate. This study has shown that each variety is susceptible to particular crack types. This may influence management practices with regard to cracking; for example, some varieties have a tendency for the economically significant side cracks, while others tend to exhibit cuticular cracks on either the apical or stylar-end of the fruit.

Once varietal susceptibility has been determined, it is important then to note that these different crack types can be exacerbated by different modes of excess water uptake. Internally driven vascular flow can increase the proportion of side-cracked fruit, and water on the surface of the fruit can increase the number of cuticular cracks. Therefore, variety and mode of water uptake must be considered together.

The two processes for excess water uptake can also occur simultaneously. Current management strategies focus on preventing water from falling on the fruit, or

drying fruit which has become wet. That two modes of water uptake are likely to result in cracking may explain why these strategies are not always effective.

The internal vascular supply of water responds to environmental conditions, and it would seem that rainfall events occurring during the afternoon following sunshine may promote higher levels of cracking. Sunshine following rainfall, however, may promote movement of water primarily through the leaves and has the potential to reduce flow to the fruit (and subsequent cracking). Irrigation is also an available source of water from which the tree can draw, and this must also be considered in cracking management.

Skin properties have been linked to crack development. Irrigation throughout the season should be managed to avoid large fluctuations in stress to ensure that fruit growth avoids patterns of excessive shrinking and swelling thereby possibly weakening the elasticity of the skin. Uniform growth will promote a well structured cuticular covering. Cuticle integrity may also be decreased through high temperatures and high light intensities.

It is therefore important to take note of seasonal conditions during the growing period, and how the climate may affect the cuticle, fruit growth and soil moisture conditions. This study has shown that seasonal cracking responses can be variable even when similar rainfall amounts were experienced.

Regardless of season and rainfall amount, perhaps the strongest possibility of cracking management is by encouraging good fruit set and retaining a high crop load. Results show increased incidence of cracking in low crop loads. Generally trees with crop loads lower than ten fruit per cm^2 TCSA showed a marked increase in cracking incidence. In terms of cracking, higher crop loads, with adequate management of carbohydrate resources, would potentially aid the reduction of fruit cracking incidence in sweet cherry. Therefore, achieving a higher crop load than ten fruit per cm^2 TCSA should be the aim. This equates to greater than 500, 750, 1000 and 2000 fruit on trees with a circumference of 25, 30, 35 and 50 cm respectively. In addition, all individual varieties monitored showed the same relationship of increased cracking with reduced crop load, and as anticipated many showed a significant relationship between crop load and the incidence of vascular-induced side cracks.

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